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GENETICS OF NATURAL POPULATIONS. VIII. CONCEALED VARIABILITY IN THE SECOND AND THE FOURTH CHROMOSOMES OF DROSOPHILA PSEUDO-OBSCURA AND ITS BEARING ON THE PROBLEM OF HETEROSIS

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INTRODUCTION

MUTATION pressure results in accumulation of genetic variability in natural populations of organisms that reproduce by cross-fertilization. The main constituents of this variability are recessive mutant genes which are carried in heterozygous condition and which are therefore concealed from direct observation and are detectable only by means of special genetic techniques. In *Drosophila pseudoobscura* a considerable proportion of wild third chromosomes contain recessive lethals and semilethals, viability and development rate modifiers, and other changes (STURTEVANT 1937; DOBZHANSKY and QUEAL 1938; DOBZHANSKY 1939; DOBZHANSKY and WRIGHT 1941; WRIGHT, DOBZHANSKY, and HOVANITZ 1942). Data to be reported in the present article show that a similar situation obtains in the second and fourth chromosomes of this species.

The determination of frequencies of major genetic changes, such as lethals, is relatively simple. Our purpose, however, has been to make an approximate census not only of major but also of minor changes—for example, modifiers of viability and of the development rate. This is extremely laborious; disregarding the preliminary experiments, we have classified and counted more than 550,000 flies. The research program of which this work is a part has been supported by a grant from the CARNEGIE INSTITUTION OF WASHINGTON, whose help we acknowledge with pleasure. We are obligated to MRS. MARIAN GIBBS HOVANITZ, MR. G. T. RUDKIN, and DR. D. D. MILLER who have shared in the work in its early stages, and to MR. E. NOVITSKI for the use of a strain containing an inversion in the second chromosome. To PROF. CARL EPLING and MR. A. SOKOLOFF we owe thanks for collecting some of the material and to DR. J. NEEL for discussion of some statistical techniques.

MATERIAL

Most of the wild flies used in the present study were collected in 1940 and 1941 in three localities, Andreas, Pinon and Keen, on Mount San Jacinto, California. A description of these localities may be found in WRIGHT, DOBZHANSKY, and HOVANITZ (1942). Population samples were taken throughout the season when the flies are reasonably abundant in

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each locality. The samples were sent to the laboratory at COLUMBIA UNIVERSITY, and the wild flies were outcrossed to the strains used for analysis either immediately or within a few days. The work on the second and on the fourth chromosomes was carried simultaneously, parts of each sample being used for both purposes. The data on the second and the fourth chromosomes are therefore fully comparable as far as the origin of the material is concerned. Some of the flies used in the experiments came from the vicinity of Santa Barbara, California (obtained through the courtesy of MR. G. MAINLAND), from the vicinity of Kaibab Lodge, Kaibab National Forest, Arizona, and from Wildrose Canyon, Panamint Mountains, California.

METHOD

The method of analysis consists of series of crosses devised to obtain individuals homozygous for wild second and wild fourth chromosomes re-

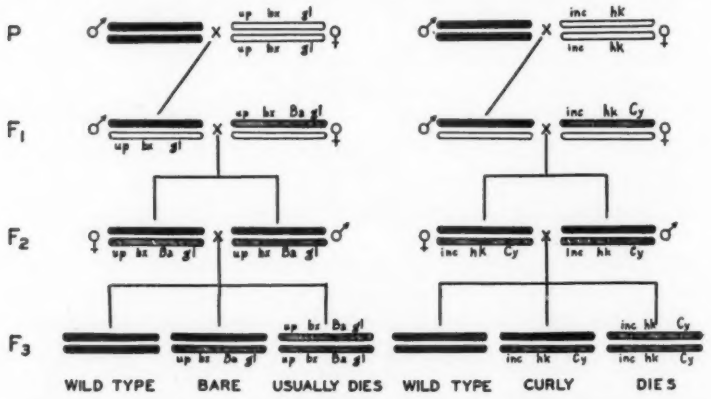

FIGURE 1.—Schemes of the crosses used to detect the recessive genetic variants in the second (left) and in the fourth (right) chromosomes of *Drosophila pseudoobscura*. Wild chromosomes are shown in black, chromosomes with marking genes in white, and chromosomes with marking genes and inverted sections hatched.

spectively (fig. 1). The recessives upturned (*upt*), bithorax (*bx*), glass (*gl*), and the dominant Bare (*Ba*) are used as markers for second chromosomes; the recessive incomplete (*inc*), hooked (*hk*), and the dominant Curly (*Cy*) are applied similarly in the fourth chromosomes. Curly is lethal to homozygotes, and homozygous Bare flies rarely survive. Inversions which suppress most of the detectable crossing over in the respective chromosomes are used when the wild chromosomes are to be transmitted through females (the F_2 generation, fig. 1). These inversions have been induced with the aid of X-ray treatments in experiments designed for that purpose; the inversion in the second chromosome was obtained by MR. E. NOVITSKI and that in the fourth chromosome by MR. G. T. RUDKIN. The strains that contain

the inversions and the dominants *Ba* and *Cy* are perpetuated in a balanced condition by means of recessive lethals in the opposite chromosomes.

To analyze the wild second chromosomes, single wild males are outcrossed to homozygous *upt bx gl* or *gl* females (fig. 1). A single F_1 male is taken from each culture and crossed to *upt bx Ba gl* inversion / lethal females. In the F_2, from six to eight females and an equal number of males showing *Ba* but not *upt bx gl* are selected; the flies are kept for two to four days in vials with food, and thereupon transferred four or five times at approximately 24 hour intervals to fresh culture bottles with the regular cornmeal-molasses-agar medium. Five or six bottles with F_2 eggs deposited in them are thus obtained. When the F_2 flies hatch, daily counts are made for a week; thereafter counts are made on alternate days until the cultures are exhausted. One of the bottles is kept in a cold room, in order to furnish material for repetition of the experiment if necessary.

The technique of analysis of wild fourth chromosomes is quite analogous to that just described for second chromosomes. Wild males are crossed to *inc hk* females, a single F_1 male is crossed to *inc hk Cy* inversion / lethal females, in the F_2 generation eight to ten females and a like number of males showing *Cy* but not *inc* or *hk* are selected, kept together in vials for two to four days, and transferred four to five times at 24 hour intervals to fresh culture bottles in which the F_2 offspring develop (fig. 1).

With the culture medium and the culture bottles used, the optimum population density for *Drosophila pseudoobscura* is between 30 and 50 flies per bottle. In our experiments the numbers of the F_2 flies per culture were so adjusted as to obtain a slight overpopulation in the F_2 bottles (100–200 flies). This was done in order to bring out more clearly any small variations in viability and development rate. The experimental cultures were kept mostly at room temperature (22–24°C). During the hot summer months they were placed in incubators at about 24½°C.

STANDARD VIABILITY AND DEVELOPMENT RATE

There is no satisfactory yardstick for measuring the absolute viability of Drosophila flies. The viability of flies homozygous for wild second and wild fourth chromosomes can therefore be measured only relative to a standard or normal viability. The duration of the development could be measured, theoretically, in days or hours, but this method is too laborious for use in practice. Consequently, we have set up a concept of normal development rate. The average viability and the average development rate of flies possessing two second, or two fourth, chromosomes taken at random from the population of a given locality are defined as normal. Since any population, excepting the extremely inbred ones, has a variety of chromosomes differing in gene contents, a majority of the flies found in nature are heterozygotes. Some flies—namely, those which have immediate an-

cestors in common—are homozygotes. The relative frequencies of hetero- and homozygotes depend upon the effective size of the population in question. To arrive at the viability and development rate standards, we must reproduce in experiments the breeding structure of natural populations. The following method of approach was used.

The wild / *upt bx Ba gl* inversion females and males from different F_2 or F_3 bottles (fig. 1) are intercrossed. Similar intercrosses are made of the wild / *inc hk Cy* inversion females and males from different strains (fig. 1). The strains intercrossed come from the same locality and preferably from flies collected at the same time. The numbers of Bare flies per culture are six to eight females and males; eight to ten Curly females and males are used in experiments involving the fourth chromosomes. In order to make the data from the intercrosses comparable to those obtained in the main experiment (in which the viability and the development rates of homozygotes are measured), the flies are permitted to oviposite for only 24 hours in each bottle. Only one culture, however, was raised for each intercross (compared to four or more for each homozygote). Counts of the flies hatching in the cultures are made at first daily, and on alternate days toward the end of the hatching period. The data for the intercrosses, which constitute our control experiment, are summarized in table 1.

TABLE 1

The viability and development rate in control cultures (further explanation in text).

DAY OF HATCHING	SECOND CHROMOSOME (BARE)				FOURTH CHROMOSOME (CURLY)			
	CULTURES WITH MORE THAN 100 FLIES		CULTURES WITH FEWER THAN 100 FLIES		CULTURES WITH MORE THAN 100 FLIES		CULTURES WITH FEWER THAN 100 FLIES	
	TOTAL FLIES	PERCENTAGE WILD TYPE	TOTAL FLIES	PERCENTAGE WILD TYPE	TOTAL FLIES	PERCENTAGE WILD TYPE	TOTAL FLIES	PERCENTAGE WILD TYPE
1	1784	31.5	1292	31.4	3817	37.0	1770	37.9
2	3270	32.7	1406	34.6	4881	35.7	1856	33.8
3	3098	33.6	659	32.0	4218	33.6	1057	29.3
4	2638	33.5	205	34.1	3353	29.2	501	25.9
5	2012	33.4	117	33.3	2504	29.7	190	25.8
6	1655	33.4	53	32.1	1645	30.2	83	22.9
7	1517	35.2	43	34.9	1098	27.7	15	26.7
8	953	32.9	13	46.2	776	30.1	4	0.0
9	776	32.6	16	18.7	456	31.8	—	—
10	517	33.8	—	—	295	27.8	—	—
11–12	1002	32.2	—	—	378	29.4	—	—
13–14	582	34.2	—	—	108	38.9	—	—
15–16	201	33.8	—	—	51	27.5	—	—
17–18	62	38.7	—	—	18	38.9	—	—
19–20	2	50.0	—	—	4	50.0	—	—
21	—	—	—	—	3	0.0	—	—
Total	20,069	33.245	3804	32.94	23,605	32.76	5476	33.07

Most of the Bare homozygotes and all Curly homozygotes die. The viability and the development rates of the wild type flies obtained in the offspring of intercrosses are normal by definition. The viability and the development rates of the Bare and the Curly flies may or may not be normal; if they are normal, the intercrosses should produce offspring consisting of wild type and of Bare or Curly flies in the ratio 33.3 percent to 66.7 percent, respectively. Any deviation from this ratio would indicate that the viability or the development rate of the Bare or Curly heterozygotes differ from the standard (fig. 2).

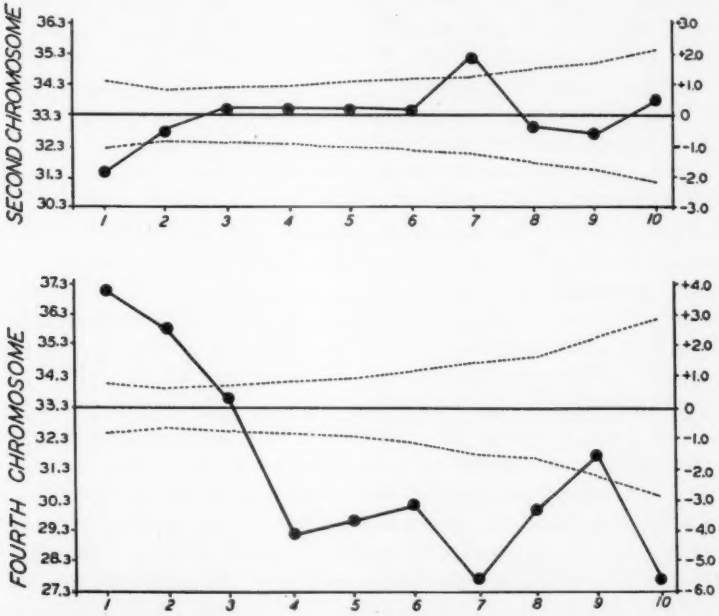

FIGURE 2.—Hatching rates of wild type individuals in the control experiments involving Bare (above) and Curly (below). Horizontal axis, days of hatching; vertical axis, percentage wild type (left) and deviation from the ideal ratio of 33.3 percent (right). Broken lines show the limits of a single standard error from the ideal ratio; the black circles and the solid lines connecting them show the observed frequencies of the wild type classes. It is obvious that in the second chromosome cultures the frequencies of wild type never deviate significantly from the ideal ratio and that in the fourth chromosome cultures the wild type class is commoner in the early than in the late hatches.

It is possible that the viability and the development rates of Bare and Curly flies are influenced by the population density in the cultures. Accordingly, the data for the cultures producing more and fewer than 100 flies are given separately in table 1. The total numbers of flies that hatched on different days in each class of cultures and the percentages of the wild

type flies among them are indicated. Examination of table 1 shows that, in the totals, the percentages of wild type flies obtained in intercrosses involving the second chromosomes are 33.24 ± 0.33 and 32.94 ± 0.76, respectively, in the cultures with more and with fewer than 100 flies. In all intercrosses involving second chromosomes, 23,873 flies have been obtained, and 33.196 ± 0.305 percent of them were wild type. It follows that flies heterozygous for the *upt bx Ba gl* inversion chromosome possess normal viability (the observed deviation from the ideal ratio of 33.333 percent is obviously not significant). For the fourth chromosomes, the cultures with more than 100 flies in each gave 32.76 ± 0.31 and those with fewer than 100 flies gave 33.07 ± 0.64 percent wild type. The totals are 29,081 flies and 32.822 ± 0.276 percent wild type. The latter figure differs by less than twice its standard error from the ideal 33.333 percent; hence, the *inc hk Cy* inversion chromosome does not affect the viability of heterozygotes.

It is important to ascertain whether the conclusion that the Bare and Curly chromosomes have no effects on the viability holds not only for the totals but also for individual cultures. It is conceivable that some of the wild strain contain modifiers which interact with Bare or with Curly in such a way as to enhance or to depress the viability of these mutants; some of the intercrosses may have carried identical second or fourth chromosomes making the wild type class homozygous rather than heterozygous (cf. p. 466). The simplest method to test the homogeneity of the results obtained in different cultures would be to compute chi-squares for each group of them and for the totals. However, for reasons which will appear later, we prefer to classify the cultures according to the magnitude of the deviation from the theoretical ratio observed in them. The theoretical ratio is, of course, 33.33 percent; the standard deviation, σ, depends upon the number of the flies obtained in each culture. The deviation observed in each culture is expressed in fractions or multiples of the standard deviation, and the cultures are classified accordingly. The total numbers of the intercrosses made are 161 for the second and 227 for the fourth chromosomes. The distribution of the cultures is shown in table 2. The expected numbers of cultures displaying deviations of various grades are calculated with the aid of tables of the normal integral of probability; these numbers are given in table 2.

The observed distribution of the second chromosome cultures agrees very closely with the expectation; the array of the intercrosses is homogeneous. The fourth chromosome cultures do not show an equally good fit (table 2); approximately six cultures have produced fewer wild type flies than they were expected to produce. Unfortunately, the particular intercrosses which gave these aberrant results could not be repeated. It may be relevant to note that in only one of the six intercrosses did one of the

TABLE 2

Deviations from the theoretical frequency of the wild type observed in the control cultures (expressed in terms of the standard deviation).

		FRACTIONS OF σ												
		−3.5	−3.0	−2.5	−2.0	−1.5	−1.0	−0.5	0	+0.5	+1.0	+1.5	+2.0	+2.5	+3.0
Second chromosomes	Cultures observed	—	—	3	8	20	24	27	27	23	17	7	4	1	
	Cultures expected	0.2	0.8	2.7	7.1	14.8	24.2	30.8	30.8	24.2	14.8	7.1	2.7	0.8	
	Difference	−0.2	−0.8	+0.3	+0.9	+5.2	−0.2	−3.8	−3.8	−1.2	+2.2	−0.1	+1.3	−0.2	
Fourth chromosomes	Cultures observed	2	5	7	14	20	30	45	39	32	17	8	4	4	
	Cultures expected	0.2	1.1	3.7	10.0	20.9	34.0	43.5	43.5	34.0	20.9	10.0	3.7	1.1	
	Difference	+1.8	+3.9	+3.3	+4.0	−0.9	−4.0	+1.5	−4.5	−2.0	−3.9	+2.0	+0.3	+2.9	

parents carry a fourth chromosome lethal, and that five out of the six cultures had fewer than 50 flies each. The fourth chromosome intercrosses may be considered to be reasonably uniform.

The development rates of the Bare and Curly flies must now be examined (table 1, fig. 2). Although the eggs in all the cultures were deposited within a period of approximately 24 hours, the hatching of the adults continued sometimes for as long as twenty days. In cultures producing fewer than 100 flies, more than 90 percent of individuals hatch within four days from the date of emergence of the first adult, and no flies appear after nine days. In cultures with more than 100 flies, only slightly more than 50 percent of the flies hatch in four days. The frequencies of the Bare and wild type flies, however, are uniform in the early and in the late hatches (fig. 2). The χ^2 for the cultures with more than 100 flies corresponds to the probability of between 0.2 and 0.1; the χ^2 for cultures with fewer than 100 flies is smaller still (probability 0.7–0.5). The conclusion follows that Bare flies have, within the limits of experimental errors, the normal development rate. Inspection of the data for individual cultures fails to disclose any intercrosses with a striking inequality of the development rates of Bare and wild type flies.

The situation is distinctly different in the intercrosses involving the fourth chromosomes (table 1, fig. 2). The frequency of the wild type class is greatest in the early hatches and drops progressively toward the end of the hatching period. As disclosed by χ^2 tests, the deviation from randomness is very significant (probability, both for cultures with more and with fewer than 100 flies, is much less than 0.01). It follows that the *inc hk Cy* inversion chromosome slows down the development of its carriers to definitely below the normal rate. It is interesting to recall that, as shown above, the same chromosome does not alter the viability.

Before completing the discussion of the control experiments, it may be noted that the fertility of the flies carrying Curly is distinctly below that of the Bare flies. Although this fact was noticed early in the work, and consequently the number of Curly parents placed in each culture bottle made greater than that of Bare parents (see above), the average output of the flies in the second chromosome control bottles turned out to be 148.3 and in the fourth chromosome bottles 128.1 flies. The Curly chromosome is completely lethal to homozygotes. The homozygous *upt bx Ba gl* flies (fig. 1) sometimes survive but are greatly delayed in hatching. A total of 483 of these homozygotes have been recorded in the intercrosses, but they are not included in table 1. The suppression of crossing over by the inversion in the Bare second chromosome is incomplete. Bare glass and upturned Bare flies appear in the cultures; 65 of the former and ten of the latter have been recorded (in table 1 and in all calculations these cross-

overs are included in the non-wild type class). The inversion associated with Curly likewise permits some crossing over to take place. Incomplete hook Curly flies appear in the cultures with a frequency close to one percent, but in our records they have not always been separated from the Curly class.

VIABILITY OF HOMOZYGOTES

The numbers of the wild second and wild fourth chromosomes analyzed are 326 and 352, respectively (table 3). In the experiments involving second chromosomes 247,010 flies were classified and counted; the corresponding figure for the fourth chromosomes is 249,468 flies. The average numbers of flies used to analyze a chromosome are therefore 757.6 for a second and 708.7 for a fourth; the numbers varied greatly from chromosome to chromosome but only very seldom did they fall below 100. Except in the preliminary experiments, the outcomes of which are included in table 4 but not in table 3, sets of at least four and sometimes of as many as twenty cultures were used to investigate the properties of each chromosome. The total output of the flies in all cultures of a set was used to characterize a given chromosome. Hence, the figures entered in tables 3 and 5 refer not to separate cultures but to chromosomes. Different cultures of a set usually agree with each other fairly well, but owing to sampling errors some disagreements have been observed. In cases of disagreement between cultures, the experiments were repeated by raising more and more cultures, until a reasonably clear decision regarding each chromosome could be reached.

The effect of the gene contents of a chromosome on the viability of the homozygotes is measured by determining the frequency of the wild type flies appearing in the cultures of F_2 or further generations (fig. 1). The control experiments (tables 1 and 2) have shown that for our purposes the viability of the Bare and Curly flies may be taken to be normal. Therefore, provided that the wild chromosomes do not modify the viability of homozygotes, the frequency of the wild type class in the cultures should be, within limits of experimental errors, close to 33.33 percent. Actually, the frequency of wild type varied from 0 to 38.14 percent for the different chromosomes analyzed (table 3).

The non-appearance of the wild type class (the zero group in table 3) indicates that the chromosome involved in a given set of cultures is lethal to homozygotes. Table 3 shows that 33 second and 35 fourth chromosomes contained lethals. Cultures with less than 4 percent wild type are rather frequent; those with from 4 to 20 percent are relatively infrequent; those with from 20 to 38 percent are common and form a skewed distribution with a mode in the 30–32 or the 32–34 percent class (table 3).

TABLE 3

Viability of types homozygous for wild second or for wild fourth chromosomes. The figures indicate numbers of strains producing various percentages of wild type.

	LOCALITY	0	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	TOTAL
Second Chromosomes	Andreas A	10	4	1	—	1	2	2	—	—	3	1	5	3	5	8	4	13	30	15	5	—	112
	Pinon A	13	3	3	2	1	—	3	1	—	1	1	—	3	3	1	10	18	38	18	2	1	122
	Keen A	1	1	1	—	—	—	—	—	1	—	1	—	1	—	—	4	7	3	5	—	—	25
	Keen D	5	2	—	1	1	—	—	—	—	—	[illegible]	1	—	—	2	3	14	11	2	—	—	43
	Santa Barbara	4	—	—	—	—	1	—	—	—	—	—	—	—	—	2	2	5	10	—	—	—	24
	Total	33	10	5	3	3	3	5	1	1	4	4	6	7	8	13	23	57	92	40	7	1	326
Fourth Chromosomes	Andreas A	2	6	4	3	—	2	2	2	2	3	1	2	6	7	19	24	18	15	4	—	—	122
	Pinon A	15	3	3	1	1	4	1	4	2	1	5	3	8	11	17	15	20	12	4	4	—	134
	Keen A	2	—	1	1	—	—	—	—	1	—	—	3	1	1	—	3	2	1	—	—	—	16
	Keen D	14	1	1	1	—	1	1	1	—	2	1	—	3	2	5	11	16	6	1	—	—	67
	Santa Barbara	2	1	2	—	—	—	—	—	—	—	—	—	—	1	—	1	1	2	2	1	—	13
	Total	35	11	11	6	1	7	4	7	5	6	7	8	18	22	41	54	57	36	11	5	—	352

A semilethal chromosome is defined as one which, in our experiments, causes death of more than half of the homozygotes. Hence, appearance of more than 0 but less than 16.7 percent wild type is evidence that the chromosome involved carries a semilethal. As many as 33 second and 54 fourth chromosomes carry semilethals (table 3). The distinction between lethals and semilethals, however, is not reliable. Some semilethal homozygotes survive very rarely, thus simulating lethals. The incomplete suppression of crossing over in the second and fourth chromosomes by the inversions used (see above) may lead to production of some wild type individuals in cultures in which the chromosome tested is completely lethal. Although semilethal homozygotes are very frequently abnormal in the phenotype, this rule has so many exceptions that it cannot be used to distinguish the lethals from the semilethals. We are confident that some of the chromosomes recorded in table 3 as extreme semilethals (the classes 0–2 and 2–4 percent) properly belong in the class of complete lethals. Figures for combined frequencies of lethals and semilethals are most reliable. Since weak semilethals (the classes with from 6 to 16.7 percent wild type) are on the whole rare, the arbitrary definition of the upper limit of semilethality does not introduce an important source of error. It may be noted in this connection that the frequencies of "lethals" given by many investigators of Drosophila genetics (particularly by students of mutation rates in *D. melanogaster*) are to be interpreted to refer to combined frequencies of lethals and semilethals, regardless of whether the authors concerned do or do not make an explicit statement to that effect.

A summary of the combined frequencies of lethals and semilethals is given in table 4. In constructing this table, we have made use not only of the data included in table 3 (which are referred to as the "main experiment") but also of the information obtained in a preliminary experiment in which only a single F_2 culture was raised to test each chromosome. Such data are satisfactory for determination of the frequencies of lethals and semilethals but not of minor viability modifiers. The apparent variations of the frequencies of lethals and semilethals from locality to locality (table 4) are not significant: the χ^2 for the second chromosomes has a probability of between 0.9 and 0.8, and that for the fourth chromosomes 0.5 to 0.3. The data, consequently, may be treated as a unit; lethals or semilethals are present in 21.27 ± 1.80 percent of the wild second and in 25.50 ± 2.17 percent of the wild fourth chromosomes tested.

The chromosomes giving more than 16.7 percent wild type must now be considered (table 3). Many of them show significant deviations from the ideal frequency of 33.3 percent wild type. Some of these chromosomes carry genetic factors that produce deteriorations or improvements of viability. Examination of the raw data on which table 3 is based (which can-

TABLE 4

Combined frequencies of lethals and semilethals (in percentage).

LOCALITY	SECOND CHROMOSOMES		FOURTH CHROMOSOMES	
	LETHALS AND SEMILETHALS	CHROMOSOMES EXAMINED	LETHALS AND SEMILETHALS	CHROMOSOMES EXAMINED
Preliminary experiment				
Wildrose	24.2	62	—	—
Andreas A	16.7	12	—	—
Pinon A	35.7	28	—	—
Keen D	19.1	89	20.0	15
Kaibab	—	—	18.9	37
Main experiment				
Andreas A	18.3	112	19.7	122
Pinon A	21.3	122	25.4	134
Keen A	16.0	25	31.2	16
Keen D	20.9	43	31.4	67
Santa Barbara	20.8	24	38.4	13
Total	21.27	517	25.50	404

not be published on account of their bulk) discloses that all sets of cultures giving less than 26 percent wild type in the total deviate from the expectation by more than three standard errors. There are 27 second and 59 fourth chromosomes of this kind (table 3). Among the sets of cultures which produce more than 26 but less than 33.3 percent wild type, there are 10 and 27 sets in the experiments involving the second and the fourth chromosomes, respectively, which deviate from the expectation by more than three standard errors. Summing up, 37 second and 86 fourth chromosomes may safely be classed as carrying genetic factors which reduce the viability of homozygotes to an extent which is perceptible but is not great enough to fall within the range of semilethality. In percentages, this means 11.3 of all the second and 24.4 of all the fourth chromosomes. Finally, some cultures have given more than 33.3 percent wild type (table 3). Among these, there are four second and two fourth chromosomes which deviate from the expectation by more than three standard errors. Thus, 1.2 percent of the second and 0.6 percent of the fourth chromosomes contain modifiers which enhance the viability of homozygotes above the standard.

The figures for the frequencies of the plus and minus viability modifiers arrived at above are patent underestimates. It is a convention that only those deviations which are three or more times greater than their standard errors are considered significant. Yet, in a normal curve 99.74 percent of the variants lie within and 0.26 percent lie outside the limits of three

standard errors from the mean. Hence, it is certain that some of the chromosomes which give deviations of less than three standard errors from the ideal ratio modify the viability of homozygotes to a perceptible extent. The problem confronting us is not so much to find out which particular chromosomes produce deteriorations or improvements of the viability as it is to estimate the frequencies of such chromosomes in the populations

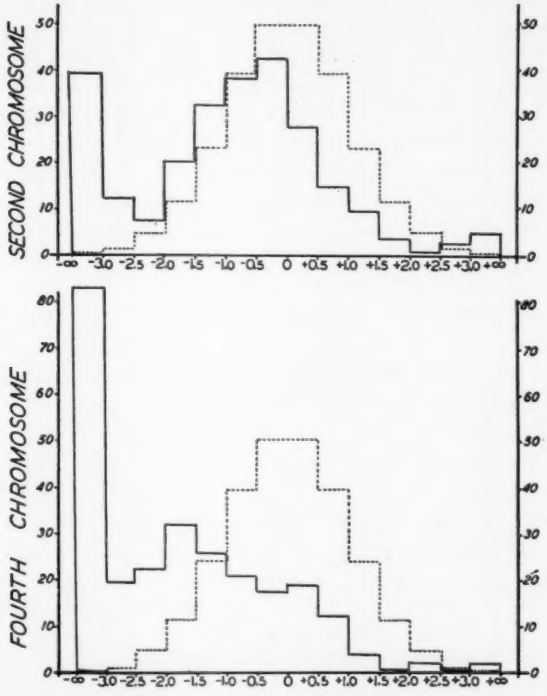

FIGURE 3.—The observed (solid lines) and the theoretical (broken lines) deviations from the ideal ratios of the wild type class. Horizontal axis, deviations expressed in fractions of σ; vertical axis, numbers of chromosomes showing these deviations.

studied. The following method is suitable for this purpose. The deviation from the ideal ratio of the wild type class is expressed in terms of fractions or multiples of the standard deviation. The deviations produced by the different chromosomes examined are entered in table 5 to form an observed distribution. With the aid of the tables of the probability integral an expected distribution is then computed. These distributions may now be compared (fig. 3). A note must be taken of the fact that in preparing table 5 we have disregarded the chromosomes that contain lethals and semi-

TABLE 5

Deviations from the theoretical frequency of the wild type class observed in the experimental sets of cultures (expressed in fractions of the standard deviation).

		FRACTIONS OF σ													
		∞	-3.0	-2.5	-2.0	-1.5	-1.0	-0.5	0	$+0.5$	$+1.0$	$+1.5$	$+2.0$	$+2.5$	$+3.0$	∞
Second chromosomes	Cultures observed	39	12	7	20	32	37	43	27	24	9	3	1	2	4	
	Cultures expected	0.3	1.3	4.3	11.5	23.9	38.9	49.8	49.8	38.9	23.9	11.5	4.3	1.3	0.3	
	Difference	+38.7	+10.7	+2.7	+8.5	+8.1	−1.9	−6.8	−22.8	−14.9	−14.9	− 8.5	−3.3	+0.7	+3.7	
Fourth chromosomes	Cultures observed	86	19	22	32	26	21	17	18	12	4	1	2	1	2	
	Cultures expected	0.3	1.3	4.3	11.6	24.2	39.4	50.4	50.4	39.4	24.2	11.6	4.3	1.3	0.3	
	Difference	+85.7	+17.7	+17.7	+20.4	+1.8	−18.4	−33.4	−32.4	−27.4	−20.2	−10.6	−2.3	−0.3	+1.7	

lethals. These chromosomes obviously do not belong to the part of the distribution shown in table 3 which centers on the 30–32 or the 32–34 percent classes. For the second chromosomes we took the ideal ratio to be 33.33 percent, and for the fourth chromosomes the ratio 32.82 percent observed in the control experiments.

The numbers of chromosomes included in table 5 are 260 and 263 second and fourth chromosomes, respectively. Comparison of the observed and the expected distributions (fig. 3) reveals that the former are definitely displaced in the minus direction. The extreme plus variants, however, are observed somewhat more frequently than they are expected to occur by chance. If superimposed on each other (fig. 3), the observed and the expected distribution curves have a large area in common, and a large area on the left and a small one on the extreme right which are covered by the observed but not by the expected distributions. This displacement of the observed with respect to the expected distribution furnishes estimates of the numbers of chromosomes that contain genetic factors depressing or enhancing the viability of homozygotes. Adding up the differences between the observed and the expected distributions shown in table 5, we find that 68.7 second and 143.3 fourth chromosomes, or 21.1 and 40.7 percent, respectively, contain unfavorable viability modifiers. On the other hand, 4.4 second and 1.7 fourth chromosomes, or 1.3 and 0.5 percent, respectively, contain favorable viability modifiers. Be it noted that even this method underestimates, rather than overestimates, the frequencies of the viability modifiers. In computing the expected distribution curves we have taken all the chromosomes not containing lethals or semilethals to be members of the distribution; actually the chromosomes giving rise to from 16.7 to 26 percent wild type are known beforehand to contain significant deviations from the normal viability.

DEVELOPMENT RATE OF HOMOZYGOTES

Since the oviposition in all the experimental cultures was restricted to about 24 hours, and since the flies hatching in them were counted daily or on alternate days, the data permit the detection of genetic factors that modify the development rate. If flies homozygous for a given wild chromosome require as much time to complete their development as normal flies do, the frequencies of the wild type class in the different hatches in any given cultures will be uniform within limits of the experimental error. If homozygotes develop more slowly than their heterozygous sibs, the frequency of wild type will be lower in the early than in the late counts. Contrariwise, acceleration of the development of homozygotes will produce a progressive decline of the frequency of wild type from the early to the late counts. In our experiments the wild type homozygotes are being com-

pared to their Bare and Curly sibs; the control experiments (table 1) have demonstrated that Bare flies have the standard development rate, while Curly develop more slowly.

Determination of the development rates of flies homozygous for wild second chromosomes is relatively simple. The numbers of wild and of all flies having hatched on the first, second, and on all subsequent days in all cultures representing a given chromosome are summed up. To decide whether or not the proportions of the wild type class observed on successive days deviate significantly from the proportion observed in the total, a chi-square test is made. If the observed or a greater deviation from homogeneity is expected to occur by chance in more than 5 out of 100 trials, the development rate is considered normal. Otherwise, the chromosome is regarded as either slowing or accelerating the development of homozygotes; the sign of the modification is, of course, determined by inspection of the data. Some development rate modifications are so striking that no statistical tests are necessary.

The chromosomes that retard the development are classified as being either slow or very slow. This classification is artificial. In cultures in which the wild type class is homozygous for a "very slow" second chromosome, the first two counts (or at least the first count if it contains more than 20 percent of the flies hatching in the given bottle) consist of Bare flies only. The "slow" class includes all significant minus deviations from the normal development rate which do not fall in the "very slow" class. Table 6 gives an illustration of the hatching rates in normal, slow, and very slow cultures. Pinon A 824 is normal, Pinon A 842 is just significantly slow (probability 0.05–0.02), and Pinon A 823 is very slow.

The numbers of the wild second chromosomes examined for viability and for development rate modifications are 326 and 274, respectively. The difference is due to the chromosomes which carry lethals and extreme semilethals being useless as far as studies on the development rates are concerned. Among the chromosomes free of lethals and extreme semilethals (table 7), 45.6 percent produce no significant modification of the development rate, 43.4 percent are slow, and 10.6 percent are very slow. A single chromosome (0.4 percent of the total) produces an acceleration of the development. The results obtained in populations of different localities are tolerably uniform.

Since Curly slows down the development of its carriers, the detection of the development rate modifiers in the wild fourth chromosomes is laborious. In many cultures the frequency of the wild type class is greatest in the early counts and diminishes in late counts. Taken at face value, such cultures may be suspected of having wild fourth chromosomes which accelerate the development of homozygotes; the suspicion is invalid unless it can be demonstrated that the wild type homozygotes develop not only

TABLE 6

Examples of modification of the development rate. The development rate of individuals homozygous for the chromosome Pinon A 824 is normal, that of Pinon A 842 homozygotes is slow, and that of Pinon A 823 homozygotes is very slow. The column marked "%" indicates the observed percentage of wild type; the column marked "n" gives the total number of flies.

CHROMOSOME	DAY OF HATCHING	NUMBERS OF THE CULTURES										TOTAL	
		NO. 1		NO. 2		NO. 3		NO. 4		NO. 5			
		%	n	%	n	%	n	%	n	%	n	%	n
Pinon A 824	1	13.3	15	0.0	4	57.1	7	33.3	27	35.3	17	30.0	70
	2	33.3	39	26.9	134	32.4	34	43.6	39	12.1	33	29.0	279
	3	38.4	52	38.0	79	22.6	31	29.0	31	25.0	44	32.5	237
	4	64.7	17	35.7	14	34.4	61	42.5	73	14.3	21	38.2	186
	5	25.0	8	—	—	33.3	27	44.4	9	0.0	1	33.3	45
	6	44.4	9	—	—	28.6	7	—	—	—	—	37.5	16
	7	33.3	6	—	—	33.3	3	—	—	—	—	33.3	9
	Total	37.0	146	30.7	231	32.4	170	39.1	179	20.7	116	32.5	842
Pinon A 842	1	33.3	3	17.9	39	25.0	4	14.3	35	0.0	3	16.7	84
	2	27.3	11	47.3	38	33.3	30	30.0	30	50.0	2	36.9	111
	3	100.0	1	0.0	1	39.0	41	37.5	40	15.0	40	30.9	123
	4	—	—	50.0	4	33.3	33	50.0	2	38.5	109	37.8	148
	5	—	—	—	—	0.0	1	46.9	32	33.3	3	44.4	36
	Total	33.3	15	32.9	82	34.9	109	32.4	139	31.9	157	32.9	502
Pinon A 823	1	0.0	12	0.0	1	0.0	3	0.0	7	0.0	2	0.0	25
	2	0.0	16	0.0	5	0.0	6	0.0	23	0.0	15	0.0	65
	3	0.0	10	4.8	21	0.0	5	0.0	47	0.0	27	0.9	110
	4	7.7	13	13.5	37	0.0	11	10.1	69	3.3	30	8.8	160
	5	3.7	27	21.9	114	2.4	41	29.6	27	10.0	50	15.4	259
	6	36.3	22	47.5	40	0.0	50	66.7	18	34.6	81	31.8	211
	7	85.7	7	54.8	31	5.9	51	100.0	10	64.8	54	46.4	153
	8	93.2	15	77.8	9	12.3	65	100.0	17	100.0	36	57.7	142
	9	85.7	7	77.8	9	43.6	103	100.0	18	83.3	12	57.7	149
	10	100.0	2	100.0	2	77.1	61	100.0	3	90.9	11	81.0	79
	11	100.0	2	100.0	1	92.8	28	—	—	—	—	93.5	31
	12	—	—	—	—	100.0	9	—	—	—	—	100.0	9
	13	—	—	—	—	100.0	1	—	—	—	—	100.0	1
	Total	30.1	133	31.1	270	32.3	434	31.4	239	39.3	318	33.3	1394

TABLE 7

Effects of wild second chromosomes on the development rate of homozygotes.

	ANDREAS A	PINON A	KEEN A	KEEN D	SANTA BARBARA	TOTAL
Normal	48	42	8	17	10	125
Slow	37	47	12	16	7	119
Very slow	10	12	2	2	3	29
Fast	1	—	—	—	—	1
Total	96	91	22	35	20	274

faster than their Curly sibs but also faster than normal flies. The control data furnish a standard of comparison (table 1). The ratios of the Curly and wild type flies to be expected on any day of hatching in cultures in which the wild type class has the normal development rate are known. The

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The chromosomes that retard the development are classified as being either slow or very slow. This classification is artificial. In cultures in which the wild type class is homozygous for a "very slow" second chromosome, the first two counts (or at least the first count if it contains more than 20 percent of the flies hatching in the given bottle) consist of Bare flies only. The "slow" class includes all significant minus deviations from the normal development rate which do not fall in the "very slow" class. Table 6 gives an illustration of the hatching rates in normal, slow, and very slow cultures. Pinon A 824 is normal, Pinon A 842 is just significantly slow (probability 0.05–0.02), and Pinon A 823 is very slow.

The numbers of the wild second chromosomes examined for viability and for development rate modifications are 326 and 274, respectively. The difference is due to the chromosomes which carry lethals and extreme semilethals being useless as far as studies on the development rates are concerned. Among the chromosomes free of lethals and extreme semilethals (table 7), 45.6 percent produce no significant modification of the development rate, 43.4 percent are slow, and 10.6 percent are very slow. A single chromosome (0.4 percent of the total) produces an acceleration of the development. The results obtained in populations of different localities are tolerably uniform.

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CHROMOSOME	DAY OF HATCHING	NUMBERS OF THE CULTURES										TOTAL	
		NO. 1		NO. 2		NO. 3		NO. 4		NO. 5		%	n
		%	n	%	n	%	n	%	n	%	n		
Pinon A 824	1	13.3	15	0.0	4	57.1	7	33.3	27	35.3	17	30.0	70
	2	33.3	39	26.9	134	32.4	34	43.6	39	12.1	33	29.0	279
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	5	25.0	8	—	—	33.3	27	44.4	9	0.0	1	33.3	45
	6	44.4	9	—	—	28.6	7	—	—	—	—	37.5	16
	7	33.3	6	—	—	33.3	3	—	—	—	—	33.3	9
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Pinon A 842	1	33.3	3	17.9	39	25.0	4	14.3	35	0.0	3	16.7	84
	2	27.3	11	47.3	38	33.3	30	30.0	30	50.0	2	36.9	111
	3	100.0	1	0.0	1	39.0	41	37.5	40	15.0	40	30.9	123
	4	—	—	50.0	4	33.3	33	50.0	2	38.5	109	37.8	148
	5	—	—	—	—	0.0	1	46.9	32	33.3	3	44.4	36
	Total	33.3	15	32.9	82	34.9	109	32.4	139	31.9	157	32.9	502
Pinon A 823	1	0.0	12	0.0	1	0.0	3	0.0	7	0.0	2	0.0	25
	2	0.0	16	0.0	5	0.0	6	0.0	23	0.0	15	0.0	65
	3	0.0	10	4.8	21	0.0	5	0.0	47	0.0	27	0.9	110
	4	7.7	13	13.5	37	0.0	11	10.1	69	3.3	30	8.8	160
	5	3.7	27	21.9	114	2.4	41	29.6	27	10.0	50	15.4	259
	6	36.3	22	47.5	40	0.0	50	66.7	18	34.6	81	31.8	211
	7	85.7	7	54.8	31	5.9	51	100.0	10	64.8	54	46.4	153
	8	93.2	15	77.8	9	12.3	65	100.0	17	100.0	36	57.7	142
	9	85.7	7	77.8	9	43.6	103	100.0	18	83.3	12	57.7	149
	10	100.0	2	100.0	2	77.1	61	100.0	3	90.9	11	81.0	79
	11	100.0	2	100.0	1	92.8	28	—	—	—	—	93.5	31
	12	—	—	—	—	100.0	9	—	—	—	—	100.0	9
	13	—	—	—	—	100.0	1	—	—	—	—	100.0	1
	Total	30.1	133	31.1	270	32.3	434	31.4	239	39.3	318	33.3	1394

TABLE 7

Effects of wild second chromosomes on the development rate of homozygotes.

	ANDREAS A	PINON A	KEEN A	KEEN D	SANTA BARBARA	TOTAL
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Fast	1	—	—	—	—	1
Total	96	91	22	35	20	274

faster than their Curly sibs but also faster than normal flies. The control data furnish a standard of comparison (table 1). The ratios of the Curly and wild type flies to be expected on any day of hatching in cultures in which the wild type class has the normal development rate are known. The

numbers of wild type flies expected to appear on different days in cultures involving tests of wild fourth chromosomes in homozygous condition may be calculated; the expected and the observed numbers are then compared with the aid of the chi-square test.

A complication arises because the viability of the wild type class homozygous for wild fourth chromosomes is frequently below normal. Wherever the viability is close to the norm (cultures giving 30 to 36 percent wild type), the control expectations (table 1) may be used directly. Depending upon whether the hatching of the flies in the experimental cultures was complete in less or in more than seven days from the emergence of the first adult, one or the other of the two columns of frequencies of wild type shown in table 1 is used in calculations. But where a given fourth chromosome produces an appreciable modification of the viability of homozygotes, the expected frequencies of the wild type class taken from table 1 have to be adjusted to the abnormal segregation observed in the experimental cultures. All these corrections and adjustments introduce sources of error in the final computations, making the data on the fourth chromosomes less reliable than those on the second chromosomes. With this reservation, we may consider the summary of the data on the fourth chromosomes given in table 8.

TABLE 8

Effects of wild fourth chromosomes on the development rate of homozygotes.

	ANDREAS A	PINON A	KEEN A	KEEN D	SANTA BARBARA	TOTAL
Normal	68	68	8	35	11	190
Slow	30	29	2	12	—	73
Very slow	5	11	2	2	—	20
Fast	4	3	—	2	1	10
Total	107	111	12	51	12	293

Among the 293 wild fourth chromosomes which were free of lethals and extreme semilethals, 64.9 percent proved to be normal, 24.9 slow, 6.8 very slow, and 3.4 percent faster than normal. The distinction between the "slow" and the "very slow" classes among the fourth chromosomes is even more arbitrary than it is in the case of second chromosomes.

RELATIONS BETWEEN VIABILITY AND DEVELOPMENT RATE

A large proportion of the wild second and wild fourth chromosomes in the natural populations examined proved to contain genetic factors which modify the viability or the development rate of homozygotes. An inquiry must now be made into the possible relationships of these variables. It does not seem unlikely that modifications of the viability and the development rates are different expressions of the same genetic factors. A type

that develops slowly may be at a disadvantage in crowded cultures compared to its more rapidly developing sibs and may therefore display a reduced survival rate. To test this possibility, the wild second and the wild fourth chromosomes engendering normal, slow, very slow, and fast development of the homozygotes have been classified according to their viability records (that is, according to the percentage of the wild type class which they produce in the cultures). The results are summarized in table 9.

TABLE 9

Relationships between viability and development rate. The figures indicate numbers of chromosomes producing various percentages of wild type.

		2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	TOTAL
Second Chromosomes	Normal	—	—	—	1	1	—	—	2	1	3	1	3	6	8	30	41	23	4	1	125
	Slow	1	—	—	—	2	—	1	2	1	1	4	2	5	13	25	42	17	3	—	119
	Very Slow	1	1	1	2	2	1	—	—	2	1	2	3	2	2	2	7	—	—	—	29
Fourth Chromosomes	Normal	—	1	1	—	3	3	2	4	5	4	13	20	22	34	39	26	10	5	—	192
	Slow	1	—	—	4	2	2	—	2	1	3	5	2	15	12	13	8	2	1	—	73
	Very Slow	1	1	—	2	—	2	1	—	1	2	—	1	1	3	2	1	—	1	—	19
	Fast	—	—	—	—	—	—	2	—	—	—	—	—	2	4	—	—	2	—	—	10

It is obvious that there is no necessary relationship between the viability and the development rate. Some semilethals develop normally, others are slow or very slow. The viability of some homozygotes which develop very slowly is normal (see, for example, the record of the chromosome Pinon A 823 reproduced in table 6). Nevertheless, the very slow chromosomes taken as a group give a distribution of viabilities which is shifted in the minus direction compared to the distributions of the normal and slow chromosomes (table 9). The data in table 9 may be used to calculate the average viabilities of homozygotes possessing different development rates. Such calculations give the following results:

Development rate	Second chromosome	Fourth chromosome
Normal	31.22 ± 0.42	27.84 ± 0.41
Slow	30.55 ± 0.50	25.96 ± 0.86
Very slow	19.48 ± 1.80	21.00 ± 2.30
Fast	—	27.00 ± 2.26

Under the conditions of the experiments the degree of prolongation of the development which we have designated as "slow" evidently does not result in an appreciable deterioration of the viability. On the other hand, the greater delays in development which fall in the "very slow" classification lead frequently, although not necessarily, to a reduction of the viability record.

Since the results scored by a chromosome in the viability and in the development rate classifications are largely independent, one may inquire what proportion of the wild second and of the wild fourth chromosomes studied are normal in both respects. Among the second chromosomes recorded in table 9 there are 125 chromosomes with a normal development rate. Among these, 98 chromosomes give frequencies of the wild type class which differ by less than three standard errors from the ideal ratio—that is, 33.3 percent. Since a total of 326 second chromosomes have been examined (table 3), the frequency of normals is only 30.0 percent. A similar computation for the fourth chromosomes shows that 111 out of 352 chromosomes are normal; the frequency of normals is 31.5 percent. These are maximum estimates of the frequencies of normal chromosomes. As pointed out above, some of the chromosomes which, in our experiments, have produced deviations of less than three standard errors from the expected 33.3 percent undoubtedly carry viability modifiers. Likewise, some chromosomes which produce development rate modifications are included in the allegedly normal class.

STERILITY

Individuals homozygous for 275 second and for 297 fourth chromosomes have been examined for fertility (lethals and extreme semilethals are obviously not subject to fertility tests). The technique of testing for fertility is as follows. About ten wild type females and males from F_3 cultures are placed together in vials for several days and then transferred to regular culture bottles. Appearance of offspring indicates that the homozygote is fertile. If no offspring appear, the test is repeated. A second failure to produce offspring is taken as proof that the homozygote is sterile. It is evident that this experimental procedure does not discriminate between sterility which affects both sexes and that which deranges only one sex. Furthermore, in some instances the effective sterility is due not to disturbances in the reproductive system itself but to extrinsic causes, such as failure of the adult flies to live long enough to accomplish mating and oviposition. As might be expected, constitutional weakness of this sort occurs chiefly among semilethal homozygotes.

The frequencies of second and fourth chromosomes making homozygotes sterile are 13.5 ± 2.1 and 8.1 ± 1.6 percent, respectively (37 and 24 sterile homozygotes have been recorded). The following figures convey some idea about correlations between sterility on one hand and viability and development rate on the other. Among the 37 second chromosomes producing sterile homozygotes, eight were semilethal; among these, three were recorded as being very slow, four as slow, and only one as having a normal development rate. Another ten sterile homozygotes had a low viability; among them three were very slow, six were slow, and one was

normal. Finally, 19 sterile homozygotes showed normal viability at least up to the time of emergence from the pupae; among them one showed very slow, nine slow, and nine normal development rates. Among the 24 sterile fourth chromosome homozygotes, nine were semilethals with slow or very slow development; ten were afflicted with a low viability but had normal development rates; four were both low in viability and either slow or very slow in development; a single sterile homozygote was apparently normal both in viability and in development rate. Thus, although sterility tends to be correlated with a lowered viability, or with a slow development, or with both, some chromosomes seem to carry specific sterility genes.

VISIBLE MUTANTS AND OTHER GENETIC VARIANTS

Surviving individuals homozygous for semilethals frequently show abnormalities in external morphology. These abnormalities are rarely of the kind which geneticists are inclined to describe as "good mutants": flies are somewhat stunted, bristles are slender or minute-like, misshapen legs, crumpled wings, etc. Aside from these changes, 11 second and five fourth chromosomes have been recorded as producing visible mutant characteristics and subnormal or normal viability. One second chromosome contained a first class mutant resembling stubbloid in *Drosophila melanogaster;* a fourth chromosome contained a dumpy-like mutant sterile when homozygous; another fourth chromosome gave a weakly chitinized abdomen which resembled a recessive mutant found previously in the third chromosome of *D. pseudoobscura.*

Mutants altering physiological characteristics of the adult were likely to be overlooked in our experiments. Two extremely interesting types, however, have been found. One, a second chromosome recessive, makes the flies extremely sensitive to etherization. A degree of etherization which produces a reversible narcosis in the Bare sibs kills every mutant homozygote. Etherization so slight that the Bare flies are merely stupefied but continue their irregular circling and jumping movements results in death of a majority of the mutant flies. It is to be noted that the viability of this mutant as measured by the Bare:wild type ratios in the cultures is only slightly below normal. Another second chromosome recessive which has a low viability (22.2 percent wild type in the cultures) and a very slow development rate, produces a grave derangement of the muscular co ordination in the homozygotes. The flies are unable to use their wings, and they walk very little because of a lack of co-ordination in the movement of the legs. When turned on the back, these flies attain the normal position with much difficulty. They are mostly immobile, their claws firmly grasping the support. The females deposit mostly sterile but also a few fertile eggs if fertilized by normal males; the males are effectively sterile. The

longevity is poor. The syndrome as a whole suggests a derangement in the central nervous system.

COMPARISON OF VARIABILITY IN DIFFERENT CHROMOSOMES

The chromosome complex of *Drosophila pseudoobscura* includes an X chromosome, three rod-shaped autosomes (the second, third, and fourth), and a dot-like autosome (the fifth). Owing to the frequent passage of the X chromosome through the hemizygous state in the male, sex-linked mutants which lower the adaptive level of the organism in its normal environment must be eliminated relatively promptly. Only neutral variants or genetic factors which express themselves differently in the two sexes could accumulate in the X chromosomes. The fifth chromosome is not yet under genetic control, but its very small size appears to preclude the possibility that it could carry much concealed variability. The concealed variability in the three large autosomes may reasonably be supposed to constitute practically the entire store of such variability available in natural populations of the species.

TABLE 10

Frequencies of chromosomes containing different types of genetic variants (in percentage).

	SECOND CHROMOSOME	THIRD CHROMOSOME	FOURTH CHROMOSOME
Lethals and semilethals	21.3±1.8	13.9±1.0	25.5±2.2
Minus modifiers of viability	21.1±2.3	30.5	40.7±2.6
Plus modifiers of viability	1.3	0.4	0.5
Minus modifiers of development rate	54.0±3.0	?	31.7±2.7
Plus modifiers of development rate	0.4	?	3.4
Sterility factors	13.5±2.1	?	8.1±1.6
Visible mutants (minimum estimate)	4.2	3.1	1.9

A summary of the data on the frequencies of different types of concealed variants in the large autosomes of *D. pseudoobscura* is presented in table 10. In this table, the figures for the second and fourth chromosomes refer to the San Jacinto populations described in the present article. The figures for the lethals, semilethals, and visibles in the third chromosomes refer to San Jacinto populations studied by WRIGHT, DOBZHANSKY, and HOVANITZ (1942) and may safely be compared with the corresponding figures for other chromosomes. The viability modifiers in the third chromosome have not been examined with the same thoroughness as in the two other autosomes. The figures in table 10 are derived from the data of DOBZHANSKY and QUEAL (1938) reexamined with the aid of the method of expressing the deviation observed in a given culture in fractions of the standard deviation (cf. tables 2 and 5 in the present article). These data

pertain not to populations of Mount San Jacinto but to those of the Death Valley region. For this reason, and also because in the experiments of DOBZHANSKY and QUEAL (1938) only one or two culture bottles were raised to test each chromosome, the figures for the third chromosomes are not entirely comparable with the rest.

The accumulation of genetic variants in natural populations is, with any given population structure, a function of mutation rates. In comparing the behavior of different chromosomes it would seem reasonable to make the following assumptions. (1) Genes which give rise by mutation to different classes of changes (that is, lethals, viability, and development rate modifiers, visibles, etc.) are distributed at random in different chromosomes. (2) The mutation rates of genes in different chromosomes are similar on the average. (3) The number of mutations that arise in each chromosome is proportional to the number of genes contained in that chromosome and, hence, to the length of the latter.

Viewed at the metaphase plate stage, the rod-shaped autosomes of *D. pseudoobscura* and the two limbs of the X chromosome are approximately equal in length. In the salivary gland cells the chromosomes, however, are distinctly unequal. The relative lengths in the salivary gland cells are presumed to be more indicative of the gene numbers than are the metaphase lengths. Preliminary estimates of the relative lengths of the chromosomes II, XR, IV, III, XL, and V based on measurements of the published drawings of these chromosomes were 10:10:8:7:5:0.5, respectively (HELFER 1941). To secure improved data, salivary gland cells showing all the chromosomes, without any of the latter being obviously more stretched than the others, were drawn with the aid of a camera lucida. The chromosomes were then measured in the drawings by the "curvometer" measuring wheel. The measurements of all the chromosomes in each cell were added together and the lengths of the separate chromosomes expressed in percentages of the total. The absolute length of the chromosomes varies greatly from cell to cell; the maximum total length of all chromosomes observed was 1440 micra. The relative lengths are more constant. The mean values obtained in the 27 measured cells are as follows (in percentage):

XL	13.53 ± 0.25	II	23.84 ± 0.41	IV	20.47 ± 0.30
XR	23.46 ± 0.41	III	17.98 ± 0.31	V	0.63

If the assumptions stated above were correct, the frequencies of genetic changes found in the second, third, and fourth chromsomes would be in a ratio approaching 1:0.75:0.86. The data do not bear out this expectation (table 10). The expected frequencies of lethals and semilethals in the three chromsomes would be 22.1, 16.6, and 19.0 percent; the observed ones are 21.3, 13.9, and 25.5 percent, respectively. The fourth chromosome shows

too high a frequency of lethals. With respect to the frequencies of the deleterious viability modifiers, the discrepancy is even more striking, the fourth chromosome again having more than its expected share. With respect to the modifiers which retard the development rate, the relations are reversed: the fourth chromosome has many fewer such variants than the second. Other classes of variants are too rare to make comparisons profitable.

The findings just stated are extremely difficult to account for. As far as the development rate modifiers are concerned, the observed discrepancy need not be taken too seriously. The technical difficulties of estimating the frequencies of the development rate modifiers in the fourth chromosome make the figure obtained for that chromosome less reliable than that obtained for the second chromosome. But there are no similar grounds on which to discount the discrepancies observed in the lethals and the viability modifiers. In fact, the possible sources of error in our experiments would all tend to produce a result opposite to that observed. The average number of flies per culture in tests of second chromosomes was greater than the corresponding figure for the fourth chromosome tests; this would tend to produce exaggeration of the effects of the second chromosome viability modifiers. The expression of Curly in heterozygotes is at times weak, approaching wild type. If any flies were misclassified, that would increase the frequency of the wild type class. The fourth chromosome of *D. pseudoobscura*, which is for the most part homologous to the IIL chromosome of *D. melanogaster*, contains rather numerous "repeat" sections; "repeats" seem to be rare in the second chromosome. Since the second and the fourth chromosomes have been derived from the same samples collected in a series of localities, the discrepancy observed cannot be due to an accidental accumulation or a loss of certain classes of changes in a local population. It appears that only a comparative study of mutation rates in the second and fourth chromosomes could shed further light on the situation.

BEARING OF THE DATA ON THEORIES OF HETEROSIS

It has been known for centuries that close inbreeding frequently results in deterioration of the stock and that outbreeding enhances the vigor (heterosis). The explanation of this phenomenon adumbrated by SHULL (1911) and developed by JONES (1917) postulates that inbreeding leads to homozygosis of concealed recessives with deleterious effects, while crossing covers up the recessives by their dominant alleles. Good evidence in favor of this view has been obtained by RICHEY and SPRAGUE (1931). EAST (1936) pointed out that heterotic effects may be produced by interaction of alleles at the same locus, but he mistakenly depreciated the importance of unmasking deleterious recessives as a consequence of inbreeding.

Studies on the genetic structure of natural populations vindicate the basic premise of the theory of SHULL, JONES, RICHEY and SPRAGUE. In the light of these studies the theory can be restated as follows. Mutation pressure introduces a certain number of mutants into the species population in every generation. The subsequent fate of the mutants depends upon their adaptive value and upon the population structure. Most mutants are in various degrees unfavorable. The dominant ones are eliminated by natural selection relatively very promptly. The recessive ones accumulate in populations of sexually reproducing and cross-fertilizing organisms until they reach equilibrium values determined by their mutation rates and by the population structure. For those mutants which are deleterious when homozygous but favorable in heterozygous condition the equilibrium values are necessarily higher than for those which are neutral in heterozygotes. In some species the effective population size is large enough, so that a great variety of chromosomes with diverse gene contents is present at all times in most breeding units. In such species it is the adaptive level of individuals heterozygous for various chromosomes which is most important, the effects of a chromosome in homozygous condition being a secondary consideration. Hence, few if any homozygotes may approach normal cross-bred individuals in vigor. In species with smaller effective populations the viability of homozygotes is important. Nevertheless, owing to the large number of loci capable of producing deleterious mutants (probably several thousand), most individuals of a species in which the average mutation rates per locus are of the order 10^{-5} will carry numerous deleterious recessive mutants. Unless the effective population size in a species is very small, its individual members to a greater or lesser extent will approach the status of balanced lethal heterozygotes.

The data in table 10 permit certain deductions concerning the status of the germ plasm in individuals of *Drosophila pseudoobscura* in natural populations. Lethals or semilethals are present in 21.3 percent of the second chromosomes. According to Hardy's formula, the frequencies of individuals carrying none, one, and two lethal or semilethal second chromosomes must be 61.9, 33.5, and 4.5 percent, respectively. Similarly, 74.1 percent of the flies will carry none, 23.9 percent one, and 1.9 percent two lethal or semilethal third chromosomes. In the fourth chromosomes, 55.5 percent of the flies will be free of lethals, and 38.0 and 6.5 percent will have, respectively, one and two lehtal or semilethal chromosomes. Disregarding the X and the fifth chromosomes, it may be computed that the percentage frequencies of flies that carry from none to six lethal or semilethal chromosomes are as follows: None, 25.5; one, 39.4; two, 25.0; three, 8.3; four, 1.5; five, 0.14; six, 0.01.

Apart from lethals and semilethals, many chromosomes carry genes which reduce the viability of homozygotes to a perceptible extent (table

10). Whether or not flies homozygous for such genes can survive and reproduce in nature is conjectural, but there is no doubt that they are discriminated against by natural selection. From the figures given in table 10 it is possible to calculate the approximate frequencies of flies carrying different numbers of chromosomes harboring such genes. These frequencies are as follows (in percentage): None, 10.6; one, 29.5; two, 33.3; three, 19.6; four, 6.2; five, 1.0; six, 0.06.

Since only 25.5 percent of the flies are free of lethals and semilethals, and only 10.6 percent are also free of the minor deleterious recessives, the frequency of flies free of both is as low as 2.7 percent. Genes that reduce the development rate of homozygotes do not necessarily cause a concomitant viability reduction under experimental conditions. Yet, the lack, or at any rate rarity, of slow types among the intercrosses indicates that in the state of nature such genes are discriminated against. The frequency of flies carrying no deleterious recessives of any kind must therefore be small indeed. The loss of vigor following inbreeding in Drosophila is understandable. It may be noted that some Drosophila stocks kept in laboratories are saved from degeneration by a selective process of which an investigator may be unaware. Thus, many strains of *D. pseudoobscura* descended from single females, caught in nature and kept in the laboratory for five and more years in small mass cultures, continue to carry two third chromosomes differing in the gene arrangement. In these strains individuals homozygous for either chromsome are probably inferior to heterozygotes.

To date, however, there is no valid reason to believe that in *D. pseudoobscura* homozygosis for every chromosome found in natural populations reduces the vigor of the strain. Flies free of deleterious recessives do occur, though rarely. Furthermore, it would seem feasible to purge the germ plasm of some strains of the viability reducers. This statement must be qualified: our methods show only the net effects on the viability of the chromosomes as wholes. As pointed out by MATHER (1941), there may exist an intra-chromosomal balance capable of releasing additional variability. In maize the situation seems to be different, at least on the surface. Inbred strains are never equal in yield to cross-bred ones, although the degree of degeneration following inbreeding varies greatly (RICHEY and SPRAGUE 1931, and others). It might be that this difference between Drosophila and maize is caused by different population structures in the two forms.

A few chromosomes (table 10) improve the viability of homozygotes compared to the standard. These chromosomes have been examined carefully and have maintained their records in repeated tests. Rarity of favorable mutants was often considered an argument against the mutation

theory of evolution; actually, it is the presence of such mutants in natural populations that demands explanation. Why have such mutants failed to become established as the wild type condition? Two answers seem possible. First, the rare chromosomes that have been observed to improve the viability in experiments may not have similar effects in the natural environment. Second, natural populations may contain mixtures of genotypes having highest adaptive values in different environments to which the species is exposed owing to climatic and micro-ecological contingencies. That the latter possibility is realized in *D. pseudoobscura* is made extremely probable by observations on seasonal changes in the frequencies of various gene arrangements in the third chromosome (unpublished data).

The technique used in the present work is contrived to detect concealed recessives, but the possibility is not excluded that some of the genetic variants found may have slight effects in heterozygotes as well. Attempts to test this possibility experimentally on third chromosome lethals gave negative results (DOBZHANSKY 1939). We have carefully examined the data on the second and fourth chromosomes which produce striking delays in the development of homozygotes (the "very slow" class, see above). It seemed possible that the heterozygous as well as the homozygous classes in such cultures take a longer time to develop than do the flies in cultures in which the chromosome tested gives a "normal" development rate in homozygotes. No indication of the existence of such dominant or semidominant changes, however, was found.

SUMMARY

Samples of natural populations of *Drosophila pseudoobscura* were taken in three localities on Mount San Jacinto, California, and in some other localities in California and Arizona. The effects on homozygotes of second and fourth chromosomes found in these samples were analyzed; 326 second and 352 fourth chromosomes were examined in detail.

Lethals and semilethals were found in 21.3 ± 1.8 percent of the second and in 25.5 ± 2.2 percent of the fourth chromosomes examined.

As many as 21.1 ± 2.3 percent of the second and 40.7 ± 2.6 percent of the fourth chromosomes reduce the viability of homozygotes to an extent which falls short of semilethality; 1.3 percent of the second and 0.5 percent of the fourth chromosomes improve the viability of homozygotes under the experimental conditions.

Homozygotes for 54.0 ± 3.0 percent of the second and 31.7 ± 2.7 percent of the fourth chromosomes have a development rate which is slower, and for 0.4 percent of the second and 3.4 percent of the fourth chromosomes a development rate which is faster than normal.

Homozygotes for at least 13.5 percent and 8.1 percent of the second and

the fourth chromosomes, respectively, are sterile. Visible mutants are carried in at least 4.2 percent of the second and 1.9 percent of the fourth chromosomes.

There seems to be no strict proportionality between the lengths of the chromosomes and the frequencies of various genetic variants found in them.

A great majority of flies in natural populations are heterozygous for one or more genes which lower the adaptive level of homozygotes. Bearings of this fact on theories of heterosis are discussed.

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THE EFFECTS OF ULTRAVIOLET AND X-RAY TREATMENT ON THE POLLEN TUBE CHROMOSOMES OF TRADESCANTIA

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THE cytogenetic interest in ultraviolet radiation resides in the hope that the qualitatively discriminating nature of this type of radiant energy will yield still further evidence as to the structure and behavior of the hereditary mechanism. It is now known that ultraviolet, like X-rays, can produce gene mutations and chromosomal changes; it is likewise known that, unlike X-rays, ultraviolet is absorbed in a definite and specific manner by the proteins and nucleic acids which constitute, as far as we are aware, the physical basis of the gene. The integration of these two kinds of investigation, the one biological and the other physico-chemical, should, in time, yield a more informative mass of data concerning the relationship between absorption on the one hand and the genic or chromosomal change on the other than the less discriminating ionizing types of radiation.

The difficulties encountered in securing and measuring the effective penetration of ultraviolet make it necessary to use a variety of organisms for genetical and cytological analyses. The studies of STADLER and SPRAGUE (1936), STADLER (1939), and MULLER and MACKENSIE (1939) have been on effects visible only after the course of many cell generations, and on those which in diploid cells are genetically viable and hence transmissible. It has been shown that such studies can be confirmed and furthered by an analysis of the more immediate effects of ultraviolet through use of the pollen tube technique (SWANSON 1940a, b), which permits an analysis of both viable and non-viable chromosomal changes before they can be eliminated through the processes of mitotic division.

MATERIAL AND METHODS

Plants are more adaptable to ultraviolet investigation than are animals because of the comparative ease of reaching the germinal material with the genetically effective wave lengths. In this study a diploid clone of *Tradescantia paludosa* Anderson & Woodson was used exclusively, the chromosomes of the generative nucleus, dividing in pollen tubes grown on an artificial sugar-agar-gelatine medium, furnishing a convenient source of material (Plate 1, A). The technique has been previously described in detail (SWANSON 1940b). In this study it was modified to the extent of re-

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placing the acenaphthene with a 0.001 percent aqueous solution of colchicine, this solution being incorporated in the medium.

The generative nuclei were irradiated two hours after the pollen grains had been dusted onto the agar medium. This, it was found, was a rather necessary procedure. Tradescantia pollen, unlike that of other plants such as maize, has a tendency to clump at the time of shedding and hence cannot be spread evenly in a monolayer. To avoid the screening effect brought about by clumping, it was necessary to delay radiation until the pollen tubes has grown out onto the surface of the medium and the generative nucleus had passed from the grain into the tube. Germination took place in fifteen minutes, and by two hours' time the majority of the tubes were in the correct stage for exposure. This treating schedule was adhered to except where noted.

Three ultraviolet sources were utilized in this study: (1) A high voltage discharge tube (Hanovia Sc-2537) operating at 5000 volts. Eighty-five percent its of ratiation is of wave length 2537. A quartz water cell was used in conjunction with this light to filter out wave length 1849 which, although present in only slight amounts, was found to be particularly injurious to the growth of pollen tubes. At 20 cm from the tube, this light gave off approximately 1000 ergs/mm²/minute. Treating distances of 10 and 20 cm were used, but the data were lumped (table 1) since the intensity factor

TABLE 1

Chromatid deletions induced by ultraviolet. High voltage discharge tube (83 percent 2536 Å).

EXPOSURE TIME	TOTAL CHR.	DELETIONS	PERCENTAGE DELETIONS
15 sec.	1124	8	.65 ± .24
30 sec.	2904	34	1.17 ± .19
1 min.	6426	174	2.71 ± .20
2 min.	4314	238	5.52 ± .34
4 min.	936	95	10.15 ± .98

was found to be non-operative. (2) A monochromator designed for the treatment of maize pollen (UBER and JACOBSOHN 1938, UBER 1940). Only wave length 2537 was used, and doses up to 3150 ergs/mm² were given. Higher doses yielded too few analyzable figures per slide. (3) A mercury arc, fitted with 1/80 M diphenyl filters so as to permit only the passage of wave lengths 2967 and 3022, and longer. At 12.5 cm from the arc, the treating distance, the energy values were 7000 ergs/mm²/minute for the shorter wave length and 21,000 ergs/mm²/minute for the longer one. These latter figures represent approximations only.

ULTRAVIOLET INDUCED ABERRATIONS

A previous study of about fifty aberrations had indicated that only single chromatid deletions resulted from the direct treatment with ultraviolet of the prophase chromosomes in the pollen tubes of Tradescantia (SWANSON 1940a). These deletions, so far as could be determined cytologically, were strictly terminal in nature. This conclusion has been made more certain by a study of more than 700 additional deletions (Plate 1, fig. B, C, D, E), all of which appear to be truly terminal. These data are in agreement with those obtained in maize where STADLER and his coworkers (STADLER 1939, SINGLETON 1939, SINGLETON and CLARK 1940) have similarly found from pachytene studies that insofar as they could determine, the deleted portion was invariably terminal. In the pollen tubes a few instances were found of sister chromatids being broken at sensibly identical loci, but in view of their scarcity, and since such sister breaks have been found as frequently in control material, it seems most probable that they are of spontaneous origin. A number of chromatid dicentrics and exchanges were also found in irradiated cells, but these, again, were no more frequent than in the controls and revealed no relation to dosage, so they must likewise be considered of a spontaneous nature.

The induced terminal deletions showed all gradations from free fragments to achromatic lesions. Most X-ray studies have disregarded aberrations of this sort because of the difficulties involved in accurate scoring, but since with ultraviolet treatment only single chromatid deletions are produced, they must be considered if any breakage data are to be obtained. Since the pollen tube technique does not permit analysis beyond the immediate cell division irradiated, it is uncertain as to whether achromatic lesions constitute true deletions. In order, therefore, to be as objective as possible in scoring, only those aberrations revealing a distinct separation between the two broken ends were considered as true deletions.

Chromatid deletions in Tradescantia pollen tubes, whether induced by X-rays or ultraviolet or arising spontaneously, are rarely dislocated from their original position. As a rule, they remain fixed in place (Plate 1, fig. C, E), held, probably, by a matrix which, as will be shown later, becomes abnormally swollen and prominent under the action of the shorter ultraviolet wave lengths. Only infrequently is the deletion moved out of place (Plate 1, fig. B, D).

Do these data agree with previous findings in other organisms? The most extensive ultraviolet investigations have been carried out by STADLER and SPRAGUE (1936) and STADLER (1939) on endosperm deficiencies in maize. These deficiencies, since they are largely of the fractional variety,

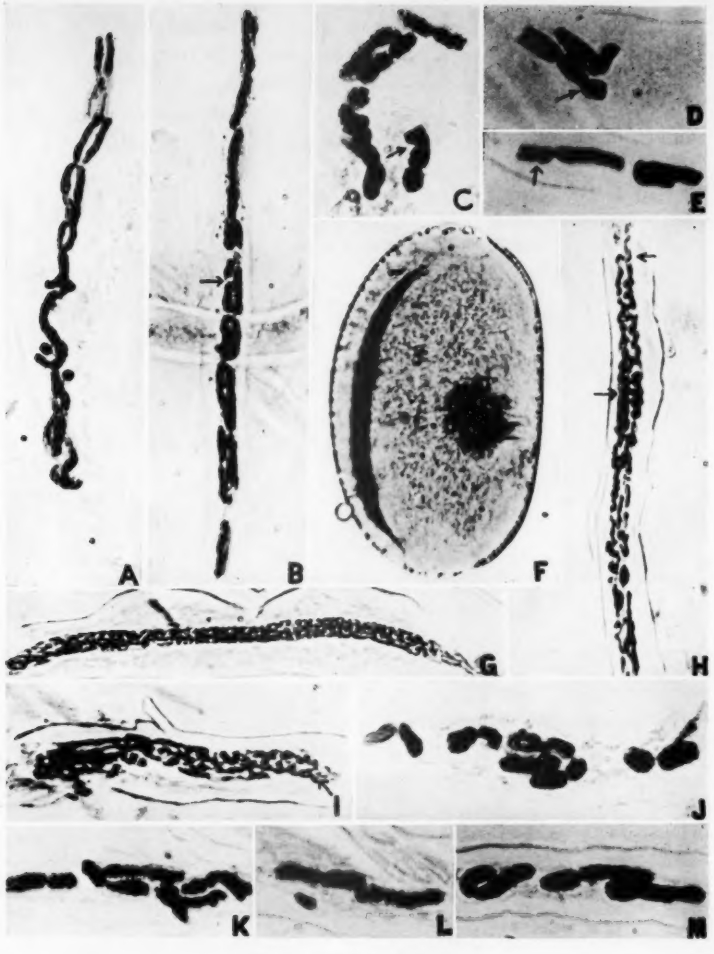
may be best explained by assuming that they represent chromatid losses comparable to those produced in Tradescantia pollen tubes. The pollen tube technique does not permit one to demonstrate that the chromatid deletions will result in segmental deficiencies of the kind shown by SINGLETON (1939) and SINGLETON and CLARK (1940), but it seems reasonable to assume that they will. STRAUB (1941) has also reported deletions (Stuckverlüste) in *Gasteria trigonia*, following treatment of mature pollen grains. These apparently terminal deletions, which STRAUB found to be not infrequent, were losses in somatic tissue, a condition which STADLER (1939) finds to be of rare occurrence in maize. An explanation for this difference in somatic losses might logically be sought in the differential capacity of the plants to survive chromatin losses, but STADLER has made it quite clear that some other explanation must be sought to clarify the situation in maize.

In addition, both STADLER and STRAUB have reported the occurrence of translocations following ultraviolet treatment. STRAUB's data seem to indicate that the translocations (five in number) were of the reciprocal type, while the three reported by STADLER were all deficiency-translocations. That the absence of translocations in the dividing generative nucleus cannot be ascribed to an insufficiency of breaks available for illegitimate reunion is evident from the data in tables 1 and 5, where X-ray and ultraviolet deletions may be compared. However, the immovability of the ultraviolet induced deletions, due possibly to the inability of ultraviolet to break through the matrix (MULLER 1940) and to the stability of such broken ends (MCCLINTOCK 1941), presages an absence of translocations at least until the release of the deletion, which in the case of the sperm nucleus would occur at the time of fertilization. This delayed formation of illegitimate reunions, following ultraviolet treatment, would parallel the delayed production of gene rearrangements in Drosophila following X-ray treatment (MULLER 1940, KAUFMANN 1941). It is, of course, possible that the occasional translocation found in the pollen tubes following irradiation is of an induced nature, but since the frequency is not above the spontaneous rate, nothing definite can be said regarding their rate of occurrence.

In Drosophila, MULLER and MACKENSIE (1939) found no chromosomal changes, either losses or rearrangements. This is perhaps due more to the lethality of even minute losses than to total absence of such changes.

RELATIONSHIP BETWEEN DOSAGE AND THE FREQUENCY OF BREAKS

STADLER and UBER (STADLER 1939) have shown that for specific endosperm deficiencies, where the induced changes are most likely the result of chromosome or chromatid deletions, the dosage curve is essentially a linear one with a leveling-off at the higher doses due to saturation effects. This



EXPLANATION OF PLATE 1

Photographs of the generative nucleus of Tradescantia.

A.—Normal metaphase.

B–E.—Ultraviolet induced deletions.

F.—Mature pollen grain showing crescent shaped generative nucleus and the diffuse tube nucleus.

G.—Generative nucleus in pollen tube two hours after germination.

H.—Generative nucleus four hours after germination. Lower arrow points to incipient somatic coils; upper arrow points to region where chromosome is split.

I.—Generative nucleus four hours after germination. Arrow indicates split chromosome.

J.—Greatly contracted chromosomes following treatment with wave length 2536; matrix not visible.

K–L.—Show development of matrices following treatment with wave-length 2536.

M.—Late prophase; matrix just beginning to show.

implies the effectiveness of single quantum absorptions. To test this further, a series of experiments were carried out to determine the dosage curve for directly induced deletions. A leveling-off should not be expected, since all deletions would be readily detected.

Table 1 and figure 1 summarize the data derived from a series of dosage

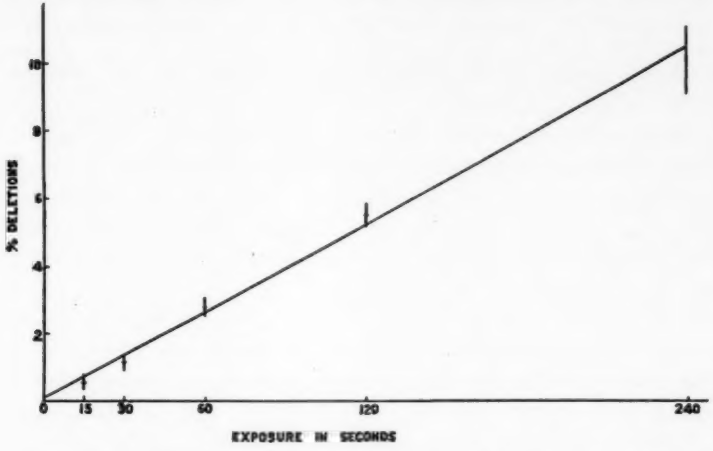


FIGURE 1.—The dosage-break relationship for simple chromatid deletions in the pollen tube chromosomes of Tradescantia at wave length 2537.

experiments employing the high voltage discharge tube. There can be little doubt of the linearity of relationship. This conclusion is further substantiated by data derived from a series of treatments using monochromatic light (wave length 2537). These are given in table 2. The errors are large,

TABLE 2

Chromatid deletions induced by ultraviolet. Monochromator. Wave length 2536.

ERGS/MM²	TOTAL CHR.	DELETIONS	PERCENTAGE DELETIONS
485	2472	19	0.77± .17
525	1008	10	0.99± .31
825	1620	7	1.66± .31
1012	282	6	2.16± .86
1050	1416	21	1.48± .32
1650	462	12	2.60± .74
2024	384	8	2.08± .73
3000	594	27	4.54± .85
3036	312	20	6.41±1.38
3150	1020	52	5.10± .69

due to the small number of observations, but the relationship is approximately linear. Can one assume, however, that the same relationship holds throughout the genetically effective spectral range, particularly in the region of the genetically weaker wave lengths? This was tentatively tested by treatments with light composed of the last two effective wave lengths (a mixture of wave lengths 2967 and 3022). The data (table 3) are meager

TABLE 3

Chromatid deletions induced by ultraviolet. Mercury arc. Mixture of wave length 2967–3022.

EXPOSURE TIME	TOTAL CHR.	DELETIONS	PERCENTAGE DELETIONS
15 sec.	1410	14	.99± .26
30 sec.	1182	15	1.27± .32
1 min.	1122	13	1.16± .32
2 min.	1200	31	2.58± .45
4 min.	462	16	3.46± .85
5 min.	216	11	5.09±1.49

and not entirely conclusive, but within the dosage used, the linearity of effect holds. The relative ineffectiveness of the longer wave lengths is indicated by a comparison with monochromatic 2537. A dose of 3000 ergs/mm^2 at wave length 2537 yields approximately 5 percent deletions. To produce the same number of deletions with a mixture of wave lengths 2967 and 3022 requires a dose of approximately 35,000 ergs/mm^2 of the former plus 100,000 of the latter. It should be emphasized, however, that these energy data are only approximate.

A comparison of the above data with those of STADLER (1939) shows that the endosperm deficiencies per unit dose of wave length 2537 are much greater in number than the terminal deletions in the generative nucleus. However, the differences in nuclei, stage of mitotic development, and methods of detection make it difficult to carry the comparison further.

THE LOCALIZATION OF CHROMATID DELETIONS

X-ray analyses of induced aberrations have revealed that the breaks are not randomly distributed along the chromosome arms. Some regions appear to be more susceptible to breakage than others. In Tradescantia microspores both X-ray and spontaneous breaks tend to have a greater proximal frequency (SAX and MATHER 1939, GILES 1940), due, according to these investigators, to the presence of a greater torsional strain imposed upon this region by the coiling mechanism. STRAUB (1941) has reported a similar distribution of breaks following both X-ray and ultraviolet treatment. X-radiation of the X chromosome of Drosophila revealed a more random break distribution, but still with a significantly higher increase

over expectancy in the heterochromatic proximal portion (BAUER, DEMEREC, and KAUFMANN 1938). KAUFMANN (1939, 1941), in a more intensive study, found that a differential break frequency obtains when particular regions are taken into consideration and concludes that this is because of the localized presence of heterochromatin. In Drosophila and Tradescantia, secondary factors, such as propinquity, movement, etc., probably play effective roles in obscuring the original frequency and distribution since the aberrations scored depend upon the existence of two breaks followed by illegitimate reunion, whether sister or non-sister. The relative absence of translocations following ultraviolet treatment indicates that illegitimate reunions are at a minimum or occur not at all in the pollen tubes. The observed break frequency should therefore more nearly reflect the original frequency, considering the stability of the broken ends so induced (MCCLINTOCK 1941).

Table 4 summarizes the data compiled on the localization of simple

TABLE 4

Localization of chromatid deletions.

REGION OF ARM*	1	2	3	4	5	TOTAL CHR.
UV (2536 Å)†	62	109	166	134	108	579
X-ray (dry pollen)	18	23	20	35	25	121
X-ray (germ. 1¾ hrs.)	20	26	45	49	42	182

* Proximal region of arm = 1; distal region = 5.

† These deletions from experiments using monochromator (2537 Å) and high voltage discharge tube (85% 2537 Å).

chromatid deletions both for ultraviolet (wave length 2537) and X-rays. The X-ray data are included for the sake of comparison and because such deletions do not depend upon subsequent reunion for their permanence and expression and should therefore reveal an original distribution. X-ray treatment of the generative nucleus in the pollen grain showed the breaks to be more or less at random, χ^2 revealing no significant deviation at the 5 percent level.

On the other hand, treatment of the generative nucleus in the pollen tube, whether with ultraviolet or X-rays, produced a greater frequency of deletions in the medial and distal regions of the chromosome arms. Calculations for χ^2 showed these departures from randomness to be statistically significant, P being less than .01. These data are directly opposite to those reported in Tradescantia microspores for more complex breaks (SAX and MATHER 1939, GILES 1940), and in Gasteria for deficiencies (STRAUB 1941). The failure of the dry pollen X-ray series to reveal other than a random distribution may be due in part to the fact that the sample

is too small to show a significant departure from randomness, since on a percentage basis the distribution is not too far different from that in the ultraviolet series, but it may also be due to the lack of secondary factors, such as torsional strains, in the passive generative nucleus prior to its passage into the pollen tube. It should be emphasized at this point that the above considerations are based on the assumption that the degree of contraction of the somatic chromosomes between prophase and metaphase is equal in all regions. However, it is recognized that a localization of breaks at metaphase can occur from a random break distribution at prophase if, during the coiling cycle, a particular region of the chromosome contracts to a greater extent than the remainder of the chromosome, a condition known to exist, for example, in the B-chromosome of maize. On the other hand, the fact that in a single genus, Tradescantia, dissimilar localizations of breaks have been found would seem to suggest that secondary factors of some sort or another are operative and that these factors may not be the same in different types of cells. As reported in a later section, the ultraviolet treatment causes a marked shortening of the chromosome, which fact might tend to obscure or accentuate a localization of breaks, but since the X-ray series (germ. 1¾ hours) follows a similar trend, it would appear that a differential contraction factor cannot be used to explain the observed distributions.

CHROMATID BREAKAGE IN RELATION TO PROPHASE DEVELOPMENT

Experimental results have consistently shown that dividing cells are more susceptible to X-rays than are those in a resting condition. Investigators, however, differ as to the most susceptible period in the cycle of cell division insofar as chromosomal breakage is concerned.

In the pollen tube it is possible to study rather accurately the effect of radiation on different stages of nuclear development by subjecting the generative nucleus to various forms of radiant energy at stated intervals after the dry grains have been dusted on the agar medium. The regularity of germination and of prophase development of the generative nucleus under artificial culture conditions permits and justifies such a procedure. In this study both X-ray and ultraviolet treatments were given.

In the X-ray series, treatments were given to the dry grains just before they were germinated and to the generative nuclei at the 2-hour and the 4-hour periods after germination. The chromosomes in the pollen grain, judging on the basis of chromatid breaks, are effectively split at least a day, and possibly two, prior to anthesis. X-ray-induced chromsome breaks had been previously reported as a result of raying mature pollen grains, and it had been concluded that all the chromosomes in the generative nucleus were not split at the time of anthesis (SWANSON 1940a, SAX and SWANSON

1941). More recent experiments show that this conclusion can no longer be considered valid, and any chromosome break showing itself in the pollen tube must be looked upon as of spontaneous origin.

The data summarized in table 5 leave no doubt that the generative

TABLE 5

*Chromosome aberrations induced by X-ray treatment. Dose 123.2 r.**

CONDITION AT TIME OF TREATMENT	TOTAL CHR.	DELETIONS	PERCENTAGE DELETIONS	DICENTRICS	EXCH.	PERCENTAGE OF TOTAL BREAKS
Dry pollen	1950	46	2.36± .34	70	15	7.48± .59
Germ. 2 hrs.	1806	181	10.00± .70	49	40	21.70± .97
Germ. 4 hrs.	456	74	16.22±1.72	11	13	24.34±2.00

* Dose given in 1 minute.

nucleus, when in the pollen grain, is considerably more resistant to X-rays than is the same nucleus two or four hours later after it has entered into the pollen tube. This is in agreement with the previous data of SAX and SWANSON (1941). Susceptibility increases as prophase advances if one considers that the nucleus enters prophase as it passes down the tube, a likely condition since the threads become progressively more chromatic and distinct (Plate 1, compare F with G, H, and I). This increased susceptibility is due largely to a continued increase in the number of terminal chromatid deletions, for it can be seen that a fall in the percentage of chromatid dicentrics and exchanges is found at the 4-hour period following a rise at the 2-hour period. The ratio of dicentrics to exchanges also varies, going from a 4.5:1 to a 1:1 ratio as prophase advances.

When successive prophase stages were treated with ultraviolet, a somewhat different situation obtains. These data are given in table 6. The dos-

TABLE 6

Ultraviolet induced deletions at successive prophase stages.

HOURS AFTER SOWING	TOTAL CHR.	DELETIONS	PERCENTAGE DELETIONS
2	1728	92	5.32±.54
3	1404	57	4.06±.52
4	1020	26	2.55±.49
5	1134	13	1.14±.31
9	1236	7	.56±.21
11	576	2	.35±.24
13	1002	2	.20±.14
15	1470	3	.21±.12
Control	2772	8	.28±.10

age (½ minute exposure, 10 cm treating distance, using high voltage discharge tube) was one which yielded an appreciable percentage of deletions but which did not seriously retard the growth of the pollen tubes. Susceptibility to ultraviolet followed a steady and continuous decline until at eleven hours after germination there was no further production of detectable chromatid deletions due to irradiation (the spontaneous rate is around 0.2 percent). The greatest drop, however, was found in the early prophase stages.

The identical conditions of the two experiments make it possible to compare, in respect to prophase susceptibility to breakage, the effects of X-rays and ultraviolet on the pollen tube chromosomes during the 2- to 4-hour stage after germination. The increase in percentage of deletions following X-ray treatment (table 5) is in direct contrast to the decline following ultraviolet treatment (table 6), further emphasizing the genetically qualitative difference between these two types of radiation (STADLER 1939). Whether a continued increase in deletions following X-radiation would result in later prophase stages is as yet undetermined, although SAX's (1941, table 1) data on Tradescantia microspores would indicate that this might be expected.

The work of SAX (1941), SAX and SWANSON (1941), KAUFMANN (1941), and others indicates that the sensitivity of cells and chromosomes to X-rays, based on breakage data, can best be accounted for on the basis of chromosome movements, propinquity of breaks, and rapidity of nuclear changes, but it is evident that these factors must play little part in determining the effect of ultraviolet treatment since a decline rather than a rise in breakage percentage must be explained. Although the data furnish no clue to the problem, it seems likely that internal physical changes in the chromosome, the nature of which are unknown but which would effect absorption, determine the reactivity of the chromosome.

THE EFFECT OF SHORT AND LONG WAVE LENGTH RADIATION ON THE MATRIX AND ON CHROMOSOME LENGTH

Apart from the production of chromatid deletions, short wave length treatment (2537 Å) has a very noticeable effect on the length of the chromosome. The greater the dose, the more contracted are the chromosomes. Under control conditions, Tradescantia pollen tube chromosomes generally possess about twenty somatic coils at metaphase, but at doses of 3000 ergs/mm², a nearly lethal dose at monochromatic 2537, the number of coils was reduced to seven to ten per chromosome. The width of the chromosome gyre was correspondingly increased. The shortness of the chromosomes was striking (Plate 1, C and J) compared to the considerable length

of the normal ones (Plate 1, A). Apparently, the effect of short wave length radiation is to increase despiralization of the coiled chromonema (SWANSON 1942), since the chromosomes at the time of radiation have already begun to coil.

Accompanying the shortening of the chromosome length, and possibly correlated with it, is the marked appearance of the matrix. This structure, rarely seen so clearly under normal conditions, takes the form of a transparent hyaline mass surrounding each chromosome (Plate 1, K and L). The matrix becomes visible for the first time at middle or late prophase (Plate 1, M) and always includes both chromatids, usually holding them so closely approximated as to simulate a single coiled structure. Frequently the chromosome matrices fuse to form a confluent mass surrounding several chromosomes, and under conditions of heavy short wave length radiation, appear to be unable to divide into chromatid matrices as would normally occur. A few of the nuclei, even after heavy doses, lack the visible appearance of the matrix, but these likewise have the shortened length and juxtaposed chromatids (Plate 1, J). This would appear to indicate that ultraviolet can cause a marked contraction of the chromosome without affecting the matrix, a condition which would mean that chromosome contraction is a phenomenon apart from, and not dependent upon the behavior of the matrix. A change in the internal condition of the chromosomes, brought about by the short wave length radiation, results in an accentuated shortening. On the other hand, it may be that the matrix is present but not visible.

Such a behavior does not take place when the longer wave lengths (a mixture of wave lengths 2967 and 3022) are used, even though the dosage in ergs may be many times as great. The chromosomes are normal in appearance. There is no marked shortening of the chromosomes, the matrix is not visibly affected, and separation of the two chromatids readily takes place. It is probable that the matrical substance absorbs highly in the short wave length region, this absorption setting up a chemical reaction or change which leads ultimately to an abnormal contraction of the chromosome and a marked appearance of the matrix.

It is too early as yet to say much concerning the nature of the absorbing components of the matrix. However, SCHULTZ (1941) has shown that the matrix of the salivary gland chromosomes of Drosophila is readily digested away by ribo-nuclease, indicating that this structure depends upon the ribo-nucleic acid linkages for its integrity. It is possible that the ribo-nucleic acids, absorbing highly in the short wave length regions, may be responsible for the striking effect of the short wave lengths on the chromosomes.

SUMMARY

In addition to extending information on the capacity of ultraviolet to produce simple breaks in pollen tube chromosomes, the present study has brought out the following new evidence:

The frequency of chromatid breaks induced by ultraviolet at 2537 Å is directly proportional to dosage. A similar situation appears to hold for the longer wave lengths (2967 and 3022), but the scantiness of the data permit of no definite conclusion.

A non-random distribution of chromatid breaks was found when nuclei in the pollen tubes were treated with ultraviolet and X-rays. Breaks were more frequent in the medial and distal regions, contrary to that previously reported in Tradescantia and Gasteria.

A delayed series of ultraviolet treatments show that the prophase chromosomes become progressively more resistant as judged from breakage data. In the 2- to 4-hour period after germination, the chromosome appears to become more susceptible to X-ray breakage, but with ultraviolet, the reverse was true.

Following heavy doses of short wave length ultraviolet treatment at prophase, the metaphase chromosomes showed a marked shortening accompanied by the appearance of a hyaline sheath surrounding them. Longer wave lengths produced no such changes.

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TRIPLOID INTERSEXUALITY IN DROSOPHILA AMERICANA SPENCER[1]

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THE intersexuality described in the genus Drosophila may be divided into two main types. One is that produced by a principal mutant gene and one or more modifiers acting on diploid individuals and changing them into intersexes. The other type of intersexuality is produced by a change in the balance between the X chromosome and autosomes, the ratio falling between that of the normal male, 1X/2A, and that of the normal female, 2X/2A.

Included in the first group is the autosomal recessive mutant "intersex" in *Drosophila simulans* (STURTEVANT 1920, 1921); females carrying this mutant become intersexual while males become sterile but remain phenotypically normal. An apparently dominant autosomal mutation in *D. pseudoobscura* turns females into intersexes (DOBZHANSKY and SPASSKY 1942). In *Drosophila virilis* two autosomal mutants are known which produce intersexuality: one of these is a recessive (LEBEDEFF 1939), the other a dominant (NEWBY 1941). It is of interest to note that all the above intersex mutants change the females into intersexes but do not affect the sex phenotype of the male.

In the second group the classical case is that of *D. melanogaster* (BRIDGES 1921; DOBZHANSKY and BRIDGES 1928; DOBZHANSKY 1930a, b). BRIDGES found that the intersexes were the offspring of triploid females and contained three sets of autosomes and two X chromosomes; they might or might not contain a Y chromosome. He concluded that the X chromosomes carried genes which tended toward the production of female characters, while the autosomes carried genes which tended toward the production of male characters; the relative proportions of autosomes and X chromosomes then determined the maleness or femaleness of the individuals. Hybrid triploid intersexes between *melanogaster* triploid females and *simulans* diploid males were produced by SCHULTZ and DOBZHANSKY (1933). These hybrid intersexes were similar to the *melanogaster* intersexes except that the testes were rudimentary. One triploid intersex has also been found in *pseudoobscura* (DOBZHANSKY and SPASSKY 1942).

It is the purpose of this paper to report on the intersexes obtained in *Drosophila americana* Spencer as offspring of triploid females. *D. americana* is a member of the *virilis* complex of Drosophila.

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MATERIALS AND METHODS

The intersex-producing strain of *D. americana* is derived from the so-called "Overton" strain, from Overton, Ohio. All flies were raised on corn-

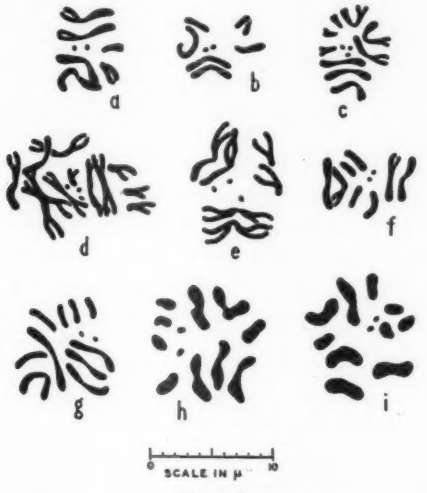

PLATE I

Metaphase chromosome configurations of different types of offspring from *D. americana* triploid females. All drawings were made with the aid of a camera lucida.

a. oogonial metaphase of diploid female; b. spermatogonial metaphase of diploid male; c. oogonial metaphase of triploid female; d. tetraploid oogonial metaphase of phenotypically diploid female; e. triplo-VI oogonial metaphase; f. oogonial metaphase of diploid female with extra Y and male-limited IV; g, h, and i. gonial metaphases of intersexual individuals.

meal-molasses Moldex food medium, the culture bottles being kept at 25°C.

Gonads of adults were smeared with aceto-carmine and the smears made permanent by the alcohol vapor-displacement method. It was found that adults 24 hours old or less were most satisfactory for obtaining metaphase chromosome pictures.

METAPHASE CHROMOSOMES OF D. AMERICANA

A knowledge of the chromosomal configurations of *americana* is important for an understanding of the triploid intersexuality in this species. Figures a and b of Plate I show that while diploid females (a) have one pair of microchromosomes, one pair of rod-shaped chromosomes, and two

pairs of V-shaped chromosomes, in the diploid male (b) there are a pair of microchromosomes, but four rod-shaped chromosomes and three V-shaped chromosomes. This peculiar condition, first observed by HUGHES (1939), becomes intelligible if the homologies with *D. virilis* are considered. *Virilis* has no V-shaped chromosomes but five pairs of rod-shaped chromosomes and a pair of microchromosomes. By different methods PATTERSON, STONE, and GRIFFEN (1940) and STALKER (1940) have shown that chromosomes II and III of *virilis* appear in a fused condition in *americana* males and females, thus accounting for the pair of V-shaped chromosomes present in both sexes. Likewise in *americana* chromosome I or X is fused with IV; the resulting V-shaped chromosome is present twice in the female but once in the male. Although *americana* X and IV are fused, Y and IV are not and appear as two rod-shaped chromosomes frequently pairing somatically with the male V-shaped X-IV.

The remaining metaphase chromosomes, the rod-shaped chromosome V and the microchromosomes, appear to be identical in *virilis* and *americana*. The scheme below will summarize these homologies.

americana female	*americana* male
X—IV II—III V VI	X—IV II—III V VI
X—IV II—III V VI	Y IV II—III V VI

TRIPLOID FEMALES

In March of 1941 a stock bottle of the Overton strain of *americana* produced a female which was aberrant in that she had roughened eyes and wing cells larger than those of her normal sisters. When mated to her brothers she produced phenotypically normal males and females, intersexes, and some females like herself. Besides the characteristics mentioned above, these aberrant females were somewhat larger and stockier than their normal sisters. Cytological examination of their ovaries showed that they were triploid, having three microchromosomes, three rod-shaped chromosomes, and six V-shaped chromosomes (Plate I, c). Clear cytological preparations of 70 phenotypically triploid females were made; with one exception (to be mentioned below), they all showed a triploid chromosome condition. In many of the preparations, particularly the later ones, the major chromosomes were present in triplicate, but only two microchromosomes were visible. This is probably due to the fact that the triploid strain had become diploid for the microchromosomes, although of course in some cases three microchromosomes might have been present but, due to their small size, might not all have been seen.

The one exceptional triploid-appearing female mentioned above showed several metaphase figures which were clearly tetraploid. This female had

enlarged wing cells and roughened eyes, and since it is very unlikely that she was a mosaic of triploid and tetraploid tissues, she was probably tetraploid throughout. BRIDGES (1925) describes several such females in *melanogaster*, although in this species they were detected by progeny tests rather than cytologically. BRIDGES suggests that such tetraploid females might arise from a 6N cyst of a triploid female; the resulting triploid egg, if fertilized by an X-bearing sperm, would form the tetraploid female.

DIPLOID MALES AND FEMALES

Large numbers of apparently normal diploid males and females were observed among the offspring of triploid females. The males rarely gave good spermatogonial metaphase figures when the testes were smeared, but of six that did give clear figures, all had the diploid male configuration (Plate I, b). Of the diploid-appearing females oogonial metaphase figures were obtained in 87 individuals, and of these, 81 showed the diploid chromosome configuration (Plate I, a), while four were diploid for the macrochromosomes, but triploid for chromosome VI, or the microchromosome (Plate I, e). It is probable that many more of the 87 females were in reality triplo-VI, but due to the small size of the microchromosomes remained undetected.

Both of the other two diploid-appearing females showed oogonial metaphase configurations which were tetraploid for the macrochromosomes, although it could not be established for certain that there were four microchromosomes present (Plate I, d). Since these females did not have enlarged wing cells or roughened eyes like the triploid females, they were in all probability diploid females with patches of tetraploid tissue which happened to include all or part of the gonads. BRIDGES (1925) reports similar patches of tetraploid tissue found in diploid females of *melanogaster* and suggests that these cells would give rise to diploid gametes, which, if they were fertilized by normal X-bearing sperm, would produce triploid females. Such tetraploid patches might well account for the many recurrences of triploidy in this species. The occurrence of patches of tetraploid tissue is apparently very common in the testes of *D. pseudoobscura* interracial hybrid males (DOBZHANSKY 1934).

Since in the *americana* male the rod-shaped Y and IV chromosomes regularly pair somatically with the V-shaped X-IV chromosome, if disjunction were normal, the X-IV would go to one pole, and the Y and IV to the other. If the disjunction were not normal, some sperm should be formed carrying both the X-IV and the Y, and some carrying the male-limited IV but no Y. The X-IV Y sperm, if they fertilized haploid eggs, would form diploid females with four V-shaped chromosomes and three rods (X-IV X-IV Y II-III II-III V V). By comparison with *melanogaster*,

such diploid females carrying a Y chromosome would be expected to be phenotypically normal and viable; in fact they make up 41 percent of the offspring of *melanogaster* triploid females (BRIDGES and ANDERSON 1925). If a diploid egg (from a triploid female) were fertilized by a X-IV Y sperm, a triploid female with six V-shaped chromosomes and four rods would result. No females of either of these two types were found.

If non-disjunction between the two fourth chromosomes occurred, some sperm would be formed which had a Y but no IV, and some with an X-IV and a male-limited IV. Sperm of the first type (Y) fertilizing eggs with one set of autosomes but two X-IV chromosomes (from triploid females)

TABLE 1*

A comparison of the chromosomal formulae of various sex-types in D. melanogaster and D. americana.

TYPE	MELANOGASTER FORMULA	AMERICANA FORMULA	AMERICANA IV/A RATIO
diploid ♂	X Y 2A	X-IV Y IV 2A	1.00
diploid ♀	2X 2A	2X-IV 2A	1.00
diploid ♀	2X Y 2A	2X-IV Y IV 2A	1.50
triploid ♀	3X 3A	3X-IV 3A	1.00
superfemale	3X 2A	3X-IV 2A	1.50
supermale	X Y 3A	X-IV Y IV 3A	.67
intersex	2X 3A	2X-IV 3A	.67
intersex	2X Y 3A	2X-IV Y IV 3A	1.00

* In this table the number of microchromosomes present is not considered.

would produce diploid females with extra Y chromosomes—that is, females having four V-shaped chromosomes and three rods.

Among the 151 phenotypically diploid and triploid females examined cytologically, none was found with a chromosome configuration indicating the presence of either a Y or an extra fourth chromosome. Thus, although non-disjunction between X-IV and Y or X-IV and IV may occur, it is probably very rare.

The fusion of *americana* X and IV and the segregation of Y and IV together in the male does not cause any unbalance between chromosome IV and the autosomes in diploid males and females, both of which have two sets of autosomes and two fourth chromosomes. However, in chromosomal type other than those of the diploid male and female, change in the X/A ratio may result in A/IV unbalance.

The first two colums of table 1 show chromosomal types BRIDGES found among the offspring of *melanogaster* triploid females. The second and third columns compare the chromosomal formulae of the various *melano-*

gaster and *americana* types. The last column of the table shows that supermales, superfemales, Y-bearing diploid females, and one type of intersex in *americana* would have an unbalance between the fourth chromosomes and the autosomes. It might be expected that such unbalance would either result in these types being very weak or absent altogether. It will be shown below that this is indeed the case; some classes of the offspring of triploid females are entirely missing, and one class is represented by a small number of very weak individuals.

DIPLOID FEMALES WITH AN EXTRA IV AND Y

Among the offspring of triploid females nine individuals were found which were phenotypically abnormal females. They had slightly spread, ventrally curved wings with slightly enlarged wing cells, and in eight out of the nine, the first section of the costal vein was shortened so that no junction was made with the first vein at the distal costal break. The heads were large with rough eyes and shortened oral palpi, the thoraces were shortened with frequently malformed legs, and the abdomina were small with unusually wide seventh sternites.

The genitalia were apparently normal with well developed ovaries. Five of the nine females gave clear oogonial metaphase preparations showing two pairs of V-shaped chromosomes, two long rods, two short rods, and a pair of microchromosomes (Plate I, f). This chromosome configuration would best be accounted for by assuming the fertilization of an egg carrying one set of autosomes and two X-IV chromosomes by a sperm with one set of autosomes, a IV and a Y. As may be seen from table 1, such females carrying an extra IV and a Y have chromosome IV in triplicate, but the other autosomes present in duplicate. If the male-limited IV is not "empty" like the Y chromosomes of *melanogaster* and *virilis*, this unbalance might be expected to cause weakness or inviability, and therefore it is not surprising that the above females were so few and so abnormal.

INTERSEXES

The gonads of several hundred intersexes were examined cytologically, and among these, 29 gave clear oogonial or spermatogonial metaphase pictures. In all 29 individuals there were five V-shaped chromosomes, five rod-shaped chromosomes, and either two or three microchromosomes. (Plate I, g, h, i). Interpreting these intersexes according to the *D. melanogaster* triploid scheme, where there are three sets of autosomes and two X chromosomes in intersexes, the five V-shaped chromosomes would be made up of three II-III chromosomes and the two X-IV chromosomes. Of the five rod-shaped chromosomes, there are three short ones and two longer ones. The three shorter ones usually show somatic pairing and are prob-

ably fifth chromosomes, the two longer ones are then the Y and the male-limited fourth chromosome, thus making up three sets of autosomes, three fourth chromosomes, and two X chromosomes. The assumption that the three short rods are fifth chromosomes and the longer ones the IV and Y gains support from the metaphase chromosome configurations of the diploid male, in which the two short rods usually show somatic pairing with each other, and the two long rods frequently pair with one of the V-shaped chromosomes.

It will be noted that all 29 intersexes carried a Y and male-limited IV, and if we ignore the fact that the microchromosomes might be present in duplicate or triplicate, they were all of one chromosomal type. As table 1 shows, two types of intersexes are known in *melanogaster*, those with a Y and those without it. But *americana* intersexes lacking a Y are hypoploid for the fourth chromosome. Since only one type of intersex has been observed in this species, the unbalance between IV and the autosomes evidently has a deleterious effect on individuals in which it occurs. Since intersexes without a IV/A unbalance survive in large numbers (see table 2), the male-limited IV presumably repairs the IV/A unbalance and thus is not "empty" like the Y of *melanogaster* and *virilis*.

TABLE 2

Proportions of different types of offspring from matings of americana triploid females×diploid males.

TYPE	NO. OBSERVED	%
diploid ♀♀	243	25.63
diploid ♂♂	295	31.12
triploid ♀♀	213	22.47
intersexes	188	19.83
diploid ♀♀+Y and ♂IV	9	.95
Total	948	

Among the offspring of the triploid females there were found no individuals which were clearly either supermales, having one X-IV and three sets of autosomes, or superfemales, with three X-IV chromosomes and two sets of autosomes. The last column of table 1 shows that supermales and superfemales would both have a IV/A unbalance. Moreover, it has been shown above that presence of an extra fourth chromosome (in diploid females) and the absence of a fourth chromosome (in one type of intersexes) results in poor viability in one case and possibly lethality in the other.

DOBZHANSKY'S (1929) work on cell size in *D. melanogaster* shows that both supermales and superfemales have wing cells larger than those of

their diploid brothers and sisters. If the same relationships hold in *americana*, then the supermales should be phenotypically distinct from their diploid brothers. They might be confused with the extreme male-type intersexes, but if present, they did not appear among the 29 intersexes examined cytologically, and the majority of these were of the extreme male type. The superfemales should have wing cells intermediate in size between those of the diploid and triploid females, yet none appeared among the 151 phenotypically diploid and triploid females examined cytologically. While it seems theoretically improbable that supersexes would survive, it is not proven that they do not come through in small numbers.

To recapitulate, three sex-types—supermales, superfemales, and one type of intersex—are found in *melanogaster* but have not been found in *americana*. The absence of these three types is presumably due to the sex-linkage of the fourth chromosome.

In *virilis* the Y chromosome apparently has no effect on the phenotype of the normal individuals, although it is known to carry the allele of the mutant sex-linked gene "bobbed." The *americana* Y chromosome is known to carry male hybrid-fertility genes (PATTERSON, STONE, and GRIFFEN 1940). Thus it may be said that the Y is "empty" not only with respect to sex-determining genes, but also as regards the large number of genes present in the X which have nothing to do with sex, but function in the ontogeny and physiology of both sexes.

Since intersexes unbalanced for the fourth chromosome are apparently inviable, we cannot test the sex determining potency of the IV by its presence or absence in intersexes. The Y and IV of *americana* could be exchanged for the Y and IV of *virilis*, in which chromosome IV is not sex-linked; but even if such a substitution produced a change in the *americana* intersexes, the result might be due to sex modifiers in the *virilis* IV which were different from those in the IV of *americana*. Such genetic modifiers of sex have been described in *melanogaster* (DOBZHANSKY 1930a) and in *virilis* (LEBEDEFF 1939).

Evidence of the presence of genes other than sex determiners in the male-limited IV is much more complete.

Salivary gland preparations of *virilis-amerianca* hybrids show that the male-limited *americana* IV is an apparently normal looking chromosome (HUGHES 1939) not largely heterochromatic like the male-limited Y-III compound chromosome of *D. miranda* (MACKNIGHT 1939).

Crosses to *virilis* have shown that the *americana* male-limited IV carries the normal allele of the *virilis* fourth chromosome recessive "plexus," the only fourth chromosome mutant available at the time the test was made (STALKER 1940).

As pointed out above, either a hypo or hyperpoloid condition of the

fourth chromosome leads to partial or complete inviability, but the male-limited IV is able to repair this unbalance.

From the above considerations it seems probable that the male-limited IV contains alleles of many if not all of the genes present in the X-linked IV. Whether it has any sex-determining potency is not known at present.

PRIMARY NON-DISJUNCTION IN AMERICANA

Primary non-disjunction of the heterochromosomes has been observed in a number of species of Drosophila, among them *Drosophila virilis* (WEINSTEIN 1922). Although no extensive tests have been made of non-disjunction in *Drosophila americana*, it may be predicted that few if any of the exceptional males or females would survive. Since the male-limited IV regularly segregates with the Y, the exceptional Y-bearing females would be triplo-IV and very weak, as shown above, while the X-IV O males would be haplo-IV, and thus would presumably not appear at all.

MORPHOLOGY OF THE INTERSEXES

The intersexual individuals are extremely variable, ranging from nearly normal male-like to fairly normal looking female-like individuals. All intersexes have irregular and enlarged eye facets and enlarged cells making up the surfaces of the wings. The intersexes have been arbitrarily divided up into six classes, in which Class I contains the most male-like individuals, Class VI the most female-like, and the intermediate classes contain the sexually intermediate forms. These six classes do not closely correspond to the seven classes of *melanogaster* intersexes distinguished by DOBZHANSKY and BRIDGES (1928). The detailed descriptions which follow are based on dissections of 186 intersexual individuals.

Class I

The individuals in this class are in general distinguishable from diploid males only by their irregular eye facets and enlarged wing cells. In this as in the other five classes of intersexes, all individuals tend to hold their wings in a slightly opened position, and in about one-quarter of the cases one or both wings are held at right angles to the longitudinal axis of the body. Approximately one-third of all intersexes have one or both of the anterior scutellar bristles missing. In most cases the posterior scutellars are more strongly convergent than in diploid and triploid individuals.

Class I intersexes have paragonia and testes that are normal in shape and position but are frequently slightly smaller than those of their normal diploid brothers. The anus has lateral plates, and the sixth and seventh tergites (Although DOBZHANSKY (1931) has presented data which indicate that it is not tergites six and seven which are fused in *Drosophila simulans*

males, this terminology will be used here to avoid confusion.) are fused as in diploid males. Claspers, penis, sperm pump, and ducts are normal. Class I individuals behave as males and court females, but they have never been observed in copula. Although when mature their ducts contain actively motile sperm, not one of the 57 individuals tested showed itself able to inseminate females.

Class II

In this class about one-fifth of the individuals show feminized anal plates —that is, instead of two lateral plates as in normal males, one of the lateral plates may be split into a dorsal and a ventral half, or there may be one entire dorsal plate and a split ventral one. The remainder of the class are like normal males in this respect.

During normal development in the male, the hypopygium, including the anal plates and external genitalia, undergoes a rotation or "circumversion" of 360° (see GLEICHAUF 1936). Since this circumversion does not occur in the female, it would be expected that some of the intersexes would have external genitalia in a somewhat asymmetrical position, between the normal male and female position. This is indeed the case, and some of the individuals of Class II show externalia rotated to various degrees.

The penis is frequently somewhat thickened and shortened, and in some individuals only one clasper is present. The sperm pump is still normal but in a few cases one testis is not attached but lies in the abdomen as a free, oval-shaped structure. In such individuals usually only one paragonium is present, being associated with the attached gonad. All Class II individuals have the sixth and seventh tergites fused as in normal males.

Class III

In this class a number of female characteristics besides the feminized anal plates put in their appearance, sometimes in individuals which also contain many male genital organs. Thus one Class III individual had an ovipositor, penis, claspers, uterus, sperm pump (at the anterior end of the uterus), spermathecae, ventral sperm receptacle, and two oviducts, which however, made no connection with the gonads.

The gonads are usually not attached to ducts, but are free orange-red oval testes, bearing no resemblance to ovaries.

Not only the anus, but frequently the external genitalia are found beneath the hypodermis in some individuals of this class. Some rotation of the anus and external genitalia is the rule rather than the exception.

In this, and Classes IV and V, the posterior end of the duct—that is, either the uterus, or ductus ejaculatorius and sperm pump—may be everted through the body wall, or more rarely through the ovipositor

plates, and appear as a black lump lying between the anus and external genitalia.

Class IV

Those individuals in which many of the genital organs of both sexes were lacking are placed in Class IV. A typical specimen of this class had no external genitalia, and the anus ended blindly beneath the surface of the hypodermis. Aside from a single oval sac-like testis lying free in the body, no genitalia of any sort, external or internal, could be found. The tergites were male-like, as in all members of Classes III and IV.

Class V

The individuals in this class are distinctly female-like but still without exception retain the male segmental condition, as tergites six and seven are fused.

Ovipositor plates are regularly present, and the anus consists of a dorsal and ventral rather than two lateral valves. Rotation of the anus and ovipositor plates is frequent; the latter are usually abnormally shortened and weakened at their anterior junction.

The uterus, spermathecae, and a ventral sperm receptacle are generally present. Short oviducts are found in about half the individuals. Three cases were noted in which spermathecae were found lying loose in the abdomen, even though the uterus and oviducts were not present. In three other cases the spermathecae lay outside the body with the everted uterus and the basal portion of the ventral receptacle.

The gonads are not spiralled as in the male types, even when they are attached. This is not surprising, since DOBZHANSKY (1931) has shown that *simulans* gynandromorph testes retain their larval ellipsoidal shape when they are attached to oviducts. The gonads are not always completely orange-red as in the three lower classes, but frequently have patches of colorless tissue. These partially colored gonads are not sac-like, but have irregular partitions which may be the beginnings of egg chambers.

Class VI

This class includes the most female-like individuals, all of which have nearly normal ovipositor-plates (although they are usually directed ventrally). A well developed uterus, ventral receptacle, spermathecae, and oviducts are also present. At least one of the gonads is an ovary with clear egg strings, although a small patch of orange-red tissue may be present at the tip. In general the segmentation is of the male type, the sixth and seventh tergites being fused. In one individual, the most female-like intersex yet observed, the segmentation on one side was male-like, while on the

other side a split had occurred which left two tergites in place of the usual 6–7 male compound. Whereas the compound male tergite has two pairs of spiracles, the split half-tergite had one spiracle below the basal part, but none below the apical half-tergite. However, a careful examination revealed that a large tracheal trunk ended blindly at the point where the missing spiracle would be expected to occur. An extra half sternite was likewise present on the same side, making the segmentation on one side that of the normal female and on the other side that of the normal male. The gonads were two normal-looking ovaries with no orange-red patches. Except for the assymetrical segmentation this specimen looked so much like a triploid female that without cytological proof of her intersexuality she would probably have been considered as an aberrant triploid. Fortunately, good oogonial metaphase figures were obtained from the ovaries, and they showed the usual intersex configuration of five V-shaped and five rod-shaped chromosomes.

Four of the seven individuals making up this class gave clear oogonial metaphase preparations. In all four, two types of chromosomes were found; those like the ones found in any normal diploid or triploid ovary and some which were so swollen as to be almost unrecognizable (Plate I, h, i). Such swollen chromosomes were found in none of the other classes of intersexes, nor in diploid or triploid individuals.

Class VI intersexes were frequently courted by diploid males.

The frequencies of the six classes of intersexes are shown in Table 3.

TABLE 3

Frequencies of different intersex classes in americana.

CLASSES	I	II	III	IV	V	VI
Number	93	50	9	10	36	8
Frequency (%)	45.1	24.3	4.4	4.8	17.5	3.9

COMPARISON OF INTERSEX MORPHOLOGY IN VARIOUS DROSOPHILA SPECIES

With a few exceptions the morphology of *americana* intersexes agrees very well with that found in *melanogaster*.

The condition of missing anterior scutellar bristles of some *americana* intersexes is not reported for intersexes in any other species of Drosophila. The holding of the wings at right angles to the body is also found in the triploid intersexes of *melanogaster* (PIPKIN 1940), and STURTEVANT (1920) finds that *simulans* males homozygous for the intersex gene also have this characteristic.

The protrusion of the attached end of the genital duct system through the hypodermis apparently does not occur in *melanogaster*, but STURTE-

VANT (1921) reports a similar phenomenon in *simulans*. However, in *simulans* the protrusion is between the ovipositor plates while in *americana* it usually takes place between the anal plate and the external genitalia.

Withdrawal of the external genitalia beneath the hypodermis, as found in *americana*, is found in the triploid intersexes of *melanogaster* as well as the diploid intersexes of *virilis* and *pseudoobscura*.

Frequently in *americana* the anal tubercle is likewise drawn beneath the hypodermis. LEBEDEFF (1939) reports that such cases in *virilis* intersexes frequently result in death, since the feces are liberated into the body cavity, there being no opening to the exterior. This is also true for *americana* intersexes.

Some intersexual individuals of both *melanogaster* and *americana* are almost totally lacking in external and internal genitalia (Class IV in *americana*), but LEBEDEFF mentions no such cases in *virilis*.

Duplication of all or part of the genitalia occurs in intersexes of *americana*, *virilis*, *melanogaster*, and *pseudoobscura*. In *americana* the externalia of both sexes may occasionally be found in one individual, but no individuals have been found with either two sets of ducts or two sets of gonads.

Virilis intersexes are reported with bisexuality of the externalia and duct systems. In some cases the two ducts lead to complete sets of gonads of opposite sex (LEBEDEFF 1939).

Pseudoobscura intersexes may have bisexual externalia and ducts but do not have more than one set of gonads, although these may differ in regard to sex.

Melanogaster triploid intersexes are externally unisexual but may (rarely) have male and female ducts in the same individual. Only one pair of gonads is found in each specimen.

While the gonads, particularly the testes of LEBEDEFF'S *virilis* intersexes, undergo internal degeneration and do not produce normal gametes, testes of the male-like intersexes of both *melanogaster* and *americana* produce sperm, and in the case of *americana*, these sperm are actively motile.

The above comparison of the triploid intersexes of *americana* with those of *melanogaster*, and the diploid intersexes of *virilis*, *simulans*, and *pseudoobscura* shows that in the more fundamental characteristics *americana* triploid intersexes are more similar to the triploid intersexes of *melanogaster* than to the diploid intersexes of *pseudoobscura*, or even the closely related species, *virilis*.

The diploid *pseudoobscura* and *virilis* intersexes agree with each other in being frequently more hermaphroditic than intersexual, while the triploid ones of *americana* and *melanogaster* rarely possess complete genital ducts or externalia of both sexes.

It is not at present clear whether or not the *americana* intersexes corrob-

orate the Time Law of Intersexuality. To clarify this point, a much larger number of intersexes should be examined.

SUMMARY

A case of triploidy in *Drosophila americana* Spencer is described.

The triploid characteristics of *americana* triploid females are phenotypically similar to those reported in *D. melanogaster*. They differ from their normal sisters in having roughened eyes, larger wing cells, and a larger body size.

Disregarding the triploidy or diploidy of the microchromosomes, six chromosomally different types of offspring were obtained from matings of *americana* triploid females by diploid males. These types were: diploid males, diploid females, triploid females, intersexes, females carrying a Y and a male-limited fourth chromosome but otherwise diploid, and tetraploid females.

Americana males and females have chromosomes X and IV fused. However, the male Y is not fused to chromosome IV. Evidence is given that disjunction of the Y and male-limited IV from the male X-IV is normal and that non-disjunction, if it occurs at all, is very rare.

All intersexes examined cytologically had the male Y and fourth chromosomes present. Intersexes without the Y would presumably be without the male-limited IV and would thus be inviable or very weak, since they would carry chromosome IV in duplicate but all the other autosomes in triplicate.

No supermales or superfemales were found. Such individuals would likewise be unbalanced for the fourth chromosome and would be expected to be inviable.

The diploid females carrying an extra Y and male-limited IV carried a duplication for the fourth chromosome (a major chromosome in *americana*). They were very weak and phenotypically abnormal.

The effect of the male-limited fourth chromosome on the intersexes and otherwise diploid females is evidence that it is not "empty," unlike the Y chromosomes of *virilis*, *melanogaster*, and many other Drosophila species.

It is pointed out that, unlike many other species of Drosophila, *americana* exceptional males and females resulting from primary non-disjunction of the heterochromosomes would be very weak in the case of the female and would probably not survive in the case of the male.

The phenotype of the intersexes is very variable, ranging from nearly normal males which produce motile sperm to fairly normal-looking females.

The intersexes morphologically resemble the triploid intersexes of *D. melanogaster* much more closely than the diploid intersexes of *D. pseudoobscura* and *D. virilis*.

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A STUDY OF A CASE OF HIGH MUTATION RATE IN DROSOPHILA MELANOGASTER

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INTRODUCTION

FROM the standpoint of evolutionary theory, as well as for the light shed on the nature of the gene, it is important to know how "spontaneous" mutation is distributed in space and time. The first adequate studies of the rate of occurrence of mutation (MULLER and ALTENBERG 1919; MULLER 1928), utilizing sex-linked lethal mutations in *Drosophila melanogaster*, served to underline this question, for whereas in the initial work, involving two different strains of flies, rates of 1.69 ± 0.46 and 0.98 ± 0.22 percent were encountered, in subsequent experiments a more normal rate of 0.19 ± 0.05 percent was observed. For some time these data remained an isolated instance of a high spontaneous mutation rate. Since 1937, however, seven additional examples of this phenomenon have been described (DEMEREC 1937; PLOUGH and HOLTHAUSEN 1937; GOLDSCHMIDT 1937, 1939; VALADARES 1937; TINIAKOV 1939; PLOUGH 1941), although one of these (GOLDSCHMIDT 1937) perhaps belongs in a separate category. In addition, SPENCER (1935) has described a cyclical variation in the frequency of mutation, involving all the strains in his laboratory. These observations have established the existence of periods of high mutational activity in Drosophila strains, although, with the exception of the case reported by DEMEREC (1937), where the phenomenon apparently depended on some recessive influence of the second chromosome, the exact causes of this mutational variability have remained obscure.

It is the purpose of the present paper to describe and analyze the cause of another case of high mutation frequency. The phenomenon was first detected in February, 1941, in two closely related strains. The first of these was a long-inbred Oregon wild type line, secured from DR. CURT STERN in 1938. During the 60 generations it had been in the author's laboratory it had been inbred in small mass cultures. Throughout this time the line had been under rather close surveillance, for it was used as a genetic background into which various bristle factors were being intro-

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duced, and crosses involving the line were rather constantly in progress. During all this time no mutants had been observed. The second line was a heterozygous *y Hw* strain which by repeated backcrosses continuing up until the discovery of the phenomenon had been rendered genetically very similar to the Oregon line. With the exception of the *y Hw* factors, the two strains were so isogenic that they may be considered as one.

THE HIGH FREQUENCY OF OCCURRENCE OF VISIBLE MUTATIONS

Between February and June, 1941, as many flies from the two lines were examined as time would permit. Whenever an off-type was found it was as a rule mated within the strain, and thus sublines of the original strain were established. The individuals comprising various sublines were likewise carefully scrutinized, and aberrant individuals bred. In this way a total of 72,851 flies was examined. The off-types found fall into three groups: (a) mutants, of which at least 47 were detected, with three more in the probable class; (b) off-types which were sterile and hence unanalyzable, but which nevertheless had the appearance of mutants—17 in number; and (c) numerous aberrant types whose unusual characteristics did not reappear in subsequent generations and which were therefore somatic mosaics or developmental accidents.

Space does not permit a description of the 47 known mutations, but a complete summary is on file with GENETICS. A summary also appears in the sixteenth issue of Drosophila Information Service. The mutations are on the whole a cross section of the types encountered in Drosophila work, with a few possible exceptions to be noted later. Thus, the sex-linked factors consisted of alleles of yellow (12), Notch (2), singed (2), bobbed (2), cut (1), carmine (1), and lozenge (1), as well as six other mutations that could not readily be homologized with any known factors. These latter six were respectively characterized by (1) a carmine-like eye color, (2) supernumerary scutellar bristles, (3) dark eyes, small body, and female sterility, (4) tiny bristles, (5) rough, oval eyes, weak wings, disarranged bristles, rarely body protuberances, and female sterility, and (6) rough eyes, missing, doubled, and disarranged hairs and bristles, and occasionally incised or blistered wings. The autosomal mutations are more difficult to homologize, but thus far have been shown to include alleles of net (1), Delta (1), and polymorph (1), and in addition factors characterized by extra bristles (3), small bristles (2), erect bristles (1), malformed wings (5) abnormal wing carriage (1), abdominal and genital imperfections (2), dark eye color (1), dark body color (1), and rough eyes (1).

A number of the mutations were characterized by small bristles (bobbed, a second sex-linked recessive, an autosomal dominant Minute, a second chromosomal recessive). These mutations became widely disseminated

throughout the strains. Toward the end of the study aberrant types with small bristles appeared with great frequency. It was obviously not feasible to test and attempt to differentiate between all these small bristle types, nor to determine which of them represented outcroppings of already isolated mutations and which new mutations. The estimate of the frequency of mutations characterized by small bristles is therefore probably low, particularly with reference to the frequency of "Minute" mutations. It should be emphasized that in this and subsequent work, the dominant autosomal Minutes were carefully distinguished from the Minute type due to the loss of the fourth chromosome through non-disjunction.

The possibility of contamination, which must always be considered, may be rigorously excluded in the great bulk of this work. The number of stocks kept in the laboratory at DARTMOUTH, where the foregoing portion of the work was carried out, was relatively small (*cf.* Drosophila Information Service #14), and few of the mutations found were duplicates of factors already present in the laboratory. Another consideration which rules out contamination is this: many of the mutations detected in the later stages of the analysis (see below) occurred in strains homozygous for one or more genetic markers. Contamination should have resulted in the disappearance or temporary suppression of these markers; this was observed in only two cases among all the thousands of cultures examined.

THE NORMAL FREQUENCY OF DETECTION OF VISIBLE MUTATIONS

For all the effort that has been expended on Drosophila, quantitative information on the normal frequency of detection of visible mutants under the conditions obtaining in such an experiment as this is surprisingly meager. Data on this point are summarized in table 1. In the hands of the average investigator a rate of about five "fair" to "good" mutants per 100,000 flies examined has been normal. After due allowance is made for the fact that some of the mutants found in the present study were "poor" ones, the mutation rate in the Oregon line (64.5 per 100,000) would on the basis of these figures appear to be at least five to ten times greater than normal, and this was the first estimate of the magnitude of the increase which the author made (NEEL 1942).

However, the existing estimates of the frequency of appearance of new mutants in *Drosophila melanogaster* are probably quite inadequate. The reasons behind this statement are chiefly two:

(1) In *ClB* experiments on induced mutations, the ratio of sex-linked lethals to visible sex-linked dominant and recessive, and autosomal dominant, mutations varies between 10 to 1 and 5 to 1, depending on the investigator. Obviously in such a case the lower ratio more nearly approximates the "true" ratio. In the later stages of the present work (see below)

TABLE 1

A compilation of the frequency of detection of visible mutants in Drosophila melanogaster.

INVESTIGATOR	ORIGIN OF FLIES EXAMINED	NUMBER FLIES	NUMBER OF MUTATIONS	RATE PER 100,000
MORGAN (1914)	?	31,168	0	0
DUNCAN (1915)	F_2 of a cross of two unrelated, inbred lines	16,637	2	12.0
GROSSMAN & SMITH (1933)	Inbred wild type stock	45,000	0	0
JOLLOS (1934)	Inbred wild type stock	ca 700,000	22	3.1
PLOUGH & IVES (1935)	Various	73,221	3	4.1
MOORE (1934)	Males from XX matings	13,673	1	7.3
	Male offspring of normal females	12,633	0	0
BUCHMANN & TIMOFEEFF-RESSOVSKY (1935)	Males from XX matings	58,453	5	8.6

a total of 7565 *ClB* F_2 vials was examined, and 25 lethals and seven visibles found. The ratio of lethals to visibles, 3.6 to 1, is sufficiently lower than the ratios encountered in irradiation work to be pertinent to the question of whether there is a difference between spontaneous and induced mutation in this respect. Be that as it may, if approximately two per 1000 is the normal rate of occurrence of sex-linked lethals, then in 100,000 *ClB* F_2 vials there should be some 200 lethals, and, on the basis of a 5 to 1 ratio of lethals to visibles, at least 40 visible mutations of the types indicated above. The *ClB* method is undoubtedly an unusually favorable means of detecting certain kinds of visible mutations. Detailed calculations, involving matters of viability, sterility, penetrance, and observational difficulties, suggest that the system of moderate inbreeding used in the work described in the previous section reduces the relative chance of detecting the average mutant to about 0.5 or so on the probability realized when the *ClB* method is employed. The *ClB* data therefore suggest that about 20 mutants due to autosomal and sex-linked dominant and sex-linked recessive factors should appear in 100,000 flies. Since autosomal recessives constitute about a third of the Drosophila mutations, with inbreeding, such as was practiced in the above described work, the expected rate of recovery of all kinds of mutations should be at least 25 per 100,000 flies. Stricter inbreeding might well result in an even higher rate of appearance of new mutations. About two thirds of these should be of the "fair" to "good" types included in table 1.

(2) Between February, 1941, and January, 1942, the period covered by this investigation, the author kept in stock an average of 20 different strains unrelated to the Oregon line and to each other. These strains were

transferred to fresh culture bottles about every 20 days, at which time some 50 flies of each strain were cursorily examined for the occurrence of contamination or mutation. Over the entire period an estimated 18,000 flies were examined. Five mutations were found, as follows:

(a) misshapen^{41d3}. 3-?. Eye variably small and deformed, overlapping with wild type.
(b) cut-out^{42a14}. Autosomal recessive. Wing margins incised. Found in same stock as (a).
(c) peach^{41d22}. 2-?. Eye color varies from orange in newly emerged flies dark, translucent prunish color in aged flies.
(d) scute^{41i}. 1–0.0. Scutellar bristles descreased in number, head bristles increased.
(e) hairy^{41k}. 3–26.5. Typical hairy-1 allele, with hairs on scutellum and along wing veins.

The occurrence of five mutations in 18,000 hastily examined flies represents a rate of 28 per 100,000, and constitutes excellent agreement with the above calculated normal rate.

The rate of visible mutation in the Oregon line was therefore two to three times greater than normal, although, as will be discussed later, this represents a minimum estimate of the increase which the factor responsible can engender.

THE FREQUENCY OF OCCURRENCE OF SEX-LINKED LETHAL MUTATIONS IN SOME STRAINS

The rate of occurrence of sex-linked lethals was investigated by the *ClB* method. In each *ClB* F_2 vial (except, of course, those indicating the presence of a lethal) an average of five males was inspected, to provide a check on the occurrence of visible mutations in the P_1 males (if all or half of the F_2 males were affected) or in the F_1 of the *ClB* cross (if only a single male was affected). Autosomal recessive mutations would not be detected by this method. The results are summarized in table 2. The first flies tested (Or F_8 1) were from the original Oregon line, seven generations after the

TABLE 2

Rate of mutation in three sublines of the frequently mutating strain.

STRAIN	NUMBER OF X CHROMOSOMES TESTED	NUMBER OF MALE P_1	SEX-LINKED LETHAL MUTATIONS	FREQUENCY OF LETHALS (%)	NUMBER OF MALE P_1 GIVING LETHALS	NUMBER OF F^2 EXAMINED (ESTIMATED)	VISIBLE P_1 MUTATIONS	VISIBLE F_1 MUTATIONS
Or F_8 1	985	46	1	0.10±0.10	1	4920	0	0
y Hw F_1 2	1021	37	8	0.78±0.28	6	5065	1	1
y Hw F_1 6	691	29	7	1.01±0.38	6	3420	1	1?

discovery of the phenomenon. The rate of occurrence of lethals appeared to be entirely normal. Two additional strains were then examined. These were selected because, as sublines, each had yielded several mutants, and, more to the point, in each a mutant had been detected quite recently. The first of these (*y Hw* F_1 2) yielded eight lethals and one visible—a new allele of prune (1–0.8)—in 1021 tested X's. Three of these lethals were derived from the same male; two of them were probably alleles, since under favorable conditions both of them behaved as semi-lethals, characterized by small, dwarf flies, but the other always behaved as a complete lethal and so probably represented a distinct mutation. Ten of the 37 males who contributed to the tested X chromosomes proved to be heterozygous for an ebony allele which had not been encountered up until this time. In one of the F_2 vials a single yellow-2 type male was found, product of a mutation in an F_1 female. In the second strain (*y Hw* F_3 6) seven lethals and one visible—characterized by a rough eye and located at 1–0.5—were found in 691 tested X's. Two of these lethals, occurring in the same male, are possibly alleles. One of the lethals, located at 1–37.2, acted under favorable conditions as a semi-lethal, resulting in flies with obliquely truncated wings and disturbed venation. The questionable F_1 visible mutation indicated in the table was a vermilion-1 (1–33.0). This factor is known to be carried within the *ClB* inversion, and it is possible that the so-called mutation was really the result of a rare double cross-over within the inversion. However, the P_1 males carried *cm* (1–18.9), and this was still present in the mutant, and l^2, also carried in the *ClB* inversion, was not associated with the vermilion.

If each lethal encountered in the latter two strains be treated as the product of a separate event, then the combined rate of lethal mutation in these two strains exceeds the rate of the Or F_8 1 strain by a significant amount. If some of the lethals are products of the same mutation, then the difference, so far as lethals alone are concerned, is less significant. However, when the occurrence of visibles in the latter two strains is also taken into account, it seems rather certain that these two strains were throwing significantly more mutations than the first strain tested. The behavior of this first strain can most reasonably be explained in terms of its loss of high mutative properties.

ANALYSIS OF THE CAUSE OF THE INCREASED MUTATION RATE

Experiment 1

A number of experiments were performed, designed to shed light on the factors responsible for the increased rate of mutation. In the first of these, an attempt was made to isolate, from the *y Hw* F_3 6 line, males producing visible mutations in relative large numbers and to establish balanced

stocks of the chromosomes of such flies. Three generations after the experiments leading to the above-described results in this strain had been started, *y Hw* F_3 6 males were mated individually with several *y S*/In(2L)*Cy*, *Cy*; *H*/In(3R)C, *Sb* females and then, three days later, remated with ca. 15 *y v f*; *bw*; *e wo ro*; *ey* females. Of 16 such attempted double matings, 13 were successful, although the fertility of the second mating was in most cases poor. The male offspring of the second cross were examined for mutant individuals. The results are summarized in table 3. Among a total of

TABLE 3

Summary of experiment 1. Explanation in text.

FLY NUMBER	NUMBER OF MALE OFFSPRING BY XX FEMALES	FERTILE MUTANTS	STERILE OFF-TYPES	MOSAICS OR DEVELOPMENTAL ACCIDENTS
1	401	0	0	0
3	202	0	0	0
4	536	1	1	4
5	1105	0	1	1
7	634	1?	0	2
8	982	0	0	2
9	297	0	0	0
10	219	0	2	1
11	382	0	0	1
12	357	0	0	0
13	408	0	0	1
14	349	0	0	0
15	284	0	0	1
13	6,156	1+1?	4	13

6156 males one certain mutation—an autosomal dominant characterized by incompletely expanded wings—and one probable mutation—resulting in a small-eyed condition—were detected. The analysis of this latter was complicated by the presence in the strain of the *ey* factor and the early loss of the strain, but the mutation was probably an autosomal dominant. The *y Hw* F_3 6 males all carried *cm* (1–18.9), which had arisen in the strain as the result of mutation, and the continued presence of which in this and subsequent experiments served as a check on contamination.

Among the 536 male offspring of male 4 there appeared, in addition to the above-mentioned certain mutant, one sterile male characterized by curled wings, rough eyes, and sparsely distributed thoracic hairs, who was in all probability the result of genetic change, and one mosaic male whose left eye was very rough, left thoracic hairs sparse and disarranged, left thoracic bristles slightly contorted, and left wing small, but whose off-type characteristics did not reappear in subsequent generations. In addition, there were three other less striking mosaics among his offspring.

On the assumption that male 4 might carry mutability-stimulating factors, an attempt was made to establish balanced stocks of his chromosomes. Accordingly, $+^m$; *S* or In(2L)*Cy*, *Cy*/$+^m$; *H* or In(3R)C, *Sb*/$+^m$ male offspring from the first cross of male 4 with *y*; *S*/In(2L)*Cy*, *Cy*; *H*/In(3R)C, *Sb* females ($+^m$ indicates the appropriate chromosome derived from the *y Hw* F_3 6 strain) were mated individually in a manner designed to balance the X chromosome against an attached-X chromosome homozygous for *y*, the second against a rearranged second chromosome with the dominant *Cy* factor in one limb and L^4 in the other (In(2L)*Cy*, *Cy*, In(2R)*Cy*, L^4 sp^2), and the third against a complexly rearranged third chromosome marked by the dominant Mé factor (T(2; 3)*Mé*). It had originally been planned to establish six or seven strains combining these balancer chromosomes with homologues derived from male 4. At a late point in the experiment it was discovered that female flies carrying all three balancers were too highly inviable and sterile to be useful. While a number of strains containing balanced first and second chromosomes derived from male 4 were secured, none completely balanced for the third was obtained. However, a number of strains with a balanced first and second, and originally heterozygous for an unbalanced third, were established, and it was also possible to establish some strains with a completely balanced first and second, and with the right end of the third balanced against In(3R)C, *Sb*, while the left end of the same chromosome remained unbalanced. No attempt was made to follow the fourth chromosome. Table 4 shows the types of balanced strains

TABLE 4

The rate of apperance of mutants in balanced stocks of the chromosomes of male 4 of experiment 1. Numbers in parentheses indicate the number of different balanced lines of the composition indicated that were followed. The question mark after certain chromosomes, or parts of chromosomes, indicates that these chromosomes may or may not have been represented in the strains.

CHROMOSOMES PRESENT IN THE STOCK	NUMBER OF FLIES EXAMINED	FERTILE MUTANTS
I, II (11)	5011	0
I, II, III? (4)	2025	1
I, II, IIIR, IIIL? (5)	2441	1
	9477	2

established, the number of flies from each examined, and the number of mutations found. The two mutations were a singed allele (1–21.0) and an autosomal recessive factor characterized by extra venation. Both of these were detected in strains in which the third chromosome of the *y Hw* F_3 6 line was either possibly or certainly represented. It is obvious that the behavior of these strains does not bear out the performance of the founder

male. The possibility that this was due to failure to control the third chromosome adequately remains open (see below).

Among the relatively few offspring of male 11 there was one off-type, a male with an abnormally oval right eye. This male, of the constitution *cm;* $+^m$*/bw;* $+^m$*/e wo ro;* $+^m$*/ey*, was mated with several *y v f; bw; e wo ro; ey* females. The oval right eye did not reappear in the next generation. However, among the 74 male offspring of the cross there was one lozenge-eyed male, subsequently found to be the result of mutation at the lozenge locus (1–27.7). It seemed possible that male 11 had actually carried a mutability stimulating factor and that its effects had not been detected because of the small number of offspring from this male. Accordingly, a balanced stock of these chromosomes was established by selecting *cm;* $+^m$*/bw;* $+^m$*/e wo ro;* $+^m$*/ey* males from the bottle which had yielded the lozenge male, crossing these with *y v f; bw; e wo ro; ey* females, and repeating the cross each generation. In order to test the frequency of mutation in these males, they were crossed with attached-X *y v f car* females, and the male offspring examined for mutants. A total of 10,084 flies was inspected. Only one mutant was detected among these—a Minute due to a dominant factor in the third chromosome—plus 11 sterile types and 22 types which bred without the reappearance of the aberrant characteristic. One of these latter was a right-left singed or forked bristle mosaic. This mutation rate did not seem to be sufficiently high to warrant further work with the strain.

Experiment 2

The second attempt to get at the factors responsible for the increased mutation rate was designed to utilize the rate of occurrence of lethal rather than visible mutation as an index of the properties of a male. Fourteen *y Hw* F_3 6 males were crossed singly with *y; S*/In(2L)*Cy, Cy; H*/In(3R)C, *Sb* females. Three days later the males were crossed with six to eight *ClB*/*y Hw*, In(1) dl–49, m^2 g^4 females. As many X chromosomes as could be obtained from each male were tested for lethals. Ten of the 14 double matings were successful. In each of the *ClB* F_2 vials an average of 10 males was examined for the occurrence of new mutants.

The results of the experiment are summarized in table 5. A number of interesting facts are obvious. First of all, the rate of lethal mutation in these ten males—at most 0.19 ± 0.08 percent—appears to be entirely normal and significantly less than what it had been in this same strain two months earlier. A χ^2 test, with Yates's correction for continuity, yields a P of less than 0.01. Secondly, the rate of visible mutation in the parental flies appears to remain high, since four visibles were found in 3103 sets of chromosomes. Two of these were sex-linked recessives—another yellow-2 type, for which male 5 was a germinal mosaic, and a tiny bristle factor

TABLE 5

Summary of experiment 2. Explanation in text.

FLY NUMBER	NUMBER OF FERTILE *ClB* VIALS	SEX-LINKED LETHAL MUTATIONS	VISIBLE P_1 MUTATIONS	NUMBER OF F_2 FLIES EXAMINED (ESTIMATED)	VISIBLE F_1 MUTATIONS	STERILE OFF-TYPES IN F_2	MOSAICS OR DEVELOPMENTAL ACCIDENTS IN F_2
3	423	0	1	4230	0	0	2
4	112	1?	0	1110	0	0	0
5	183	1	1	1820	0	1	3
6	225	0	1	2250	0	4	1
9	137	0	0	1370	0	0	0
10	558	3	0	5550	0	3	3
11	212	0	0	2120	0	2	0
12	491	0	0	4910	2	3	4
13	419	0	1	4190	3+1?	1	3
14	343	1	0	3420	0	0	1
10	3,103	5+1?	4	30,970	5+1?	14	17

(1–19.5) found in male 3—and the other two were "poor" autosomal dominants—one characterized by extra anterior scutellar bristles, and the other by slightly small, wavy-surfaced wings, which were often carried at an angle with the body. The dominant autosomal mutations may have occurred in either the male or female parents. Thirdly, among the approximately 30,970 F_2 males examined, six mutants were encountered as single individuals in different vials. All of these were found in the progeny of two males. Male 12 yielded two autosomal dominant Minute mutations, one of which was localized to the third chromosome, while male 13 yielded two autosomal dominant Minutes, one second chromsome semi-dominant characterized by curly wings, and a vermilion allele (1–33.0). As previously, the possibility exists that the vermilion appeared as the result of a double cross-over involving the *ClB* inversion. The continued presence of *cm* and the non-appearance of t^2 in the strain again provide some evidence against such an origin. The problem of determining whether or not the observed distribution of mutation in the offspring of these ten males is random is a thorny issue. However, calculations based upon any one of several different methods suggest that the "clumping" of visible mutations in the descendants of males 12 and 13 is significant. Fourthly, the occurrence of visible mutations in the F_2 of certain males would suggest that if the factors involved were genetic, they were dominant.

The chromosomes of males 12 and 13 were saved for study of their properties, using a modification of the method described under experiment 1, which made it possible to secure some lines with a completely balanced

third chromosome. The attached-X chromosome used in this experiment was *y v f*. Table 6 shows the composition of the balanced strains estab-

TABLE 6

The rate of appearance of mutants in balanced stocks of the chromosomes of males 12 and 13 of experiment 2. Numbers in parentheses indicate the number of different lines of the composition indicated that were followed. The question mark after certain chromosomes indicates that these chromosomes may or may not have been present in the strain.

CHROMOSOMES PRESENT IN THE STOCK	NUMBER OF FLIES EXAMINED	FERTILE MUTANTS
I, II (12)	13,001	2
I?, II, III (4)	4,619	0
I?, II?, III (2)	1,441	4
I?, III (8)	10,203	7
	29,264	13

lished, the number of flies examined, and the mutations found. Among a total of 29,264 flies there were 13 different mutants. These include a hairy-2 allele (3–26.5), two forked alleles (1–56.7), a singed allele (1–21.0), a yellow-1 and two yellow-2 types (1–0.0), a third chromosome recessive characterized by plexate wings, a sex-linked rough eye, and four mutants due to as yet unlocalized factors, characterized respectively by plexate wings; short, blunt bristles; Minute-like bristles; and abnormal abdomen. Only two of the mutants were detected among the 13,001 offspring of flies with balanced first and second chromosomes, while among 16,263 offspring of flies with a balanced third, which might also have a first or second from that line, there were 11 mutants. The probability that these two series have the same mutation rates, as tested by χ^2 with Yates's correction for continuity, is less than 0.01. These results indicate a role of the third chromosome or, less probably, of a combination of the first and third, in the increased mutation rate. Since, moreover, that chromosome was carried in the heterozygous state in the bulk of the flies and since the strain was perpetuated by the heterozygous flies, the influence was apparently dominant.

It should be emphasized that the presence of the various genetic markers —*y*, *v*, and *f* in the attached-X females, and *Cy*, L^4, and *Mé* in the balancing chromosomes of both males and females, together with some other markers introduced to follow certain chromosomes—tended to interfere with the detection of mutants, not only because they rendered the detection of mutants more difficult, but also because they lowered the viability and fertility of the flies to such an extent that the occurrence of further mutations might make the fly inviable or sterile.

Experiments 3 and 4

Two further experiments were undertaken to get additional information on the rate of lethal mutation. At this time there were on hand some 50 sublines of the high mutation strain. Each generation, when these lines were transferred to fresh bottles, they were given a quick inspection, and a record was kept of any mutant individuals encountered. In this way two lines were isolated which seemed to be particularly "active." The first line (*y Hw* F_3 2) had the following history: On March 9, 1941, a female with a notched right wing was found during a count of the third generation of the high mutability strain. She bred as if heterozygous for a factor designated as Notch 264–130 (1–3.0). On May 4 the subline established from her yielded two flies with slightly plexate wings, heterozygous for a new semi-dominant factor, net^{41c4} (2–0.3). On July 31, during the course of a cross to localize this factor, a dumpy allele (2–13.0) was found, but it was not clear whether this had been present in the *y Hw* F_3 2 strain or had been introduced by the second strain involved. Likewise, on August 14, in the F_2 of an outcross made for the purpose of studying the salivary gland cell chromosomes of the net flies, one of the two strains involved was found heterozygous for a recessive autosomal factor characterized by rough eyes. On September 30 two new mutants were discovered in the same bottle. One of these, characterized by very small bristles, a tarsus-like antenna, and poor fertility, was found to be due to a recessive spineless-aristopedia allele (3–58.5). The other was the resultant of a new cut allele (1–20.0). Exclusive of the original Notch, there were thus three to five mutations in about 2,000 flies.

The second line (Or F_7 2) was derived from two male and three female flies with a medially-placed dark patch on the thoracic dorsum, found in the seventh generation of the count. The character was due to an autosomal recessive. On July 17 two males with small, rough eyes were found; they were due to a new sex-linked recessive factor which was also characterized by the complete sterility of the homozygous females. August 8 the strain yielded a yellow-2 type male, and on September 11 a male with garnet-like eyes, who was sterile. Finally, on September 30 a male with tiny bristles was discovered, the result of an autosomal recessive mutation. Exclusive of the original character, there were thus three to four mutants in about 1200 flies.

The results of *ClB* tests of these two strains are summarized in table 7. There were no lethals nor visibles in a total of 849 X chromosomes examined from the *y Hw* F_3 2 strain; among ca. 8490 F_2 males two F_1 mutants were found—a Minute due to a dominant factor in the second chromosome and a spread wings due to a sex-linked recessive. However, two of the P_1 males were heterozygous for a new recessive factor in the second chromo-

TABLE 7

Summary of experiments 3 and 4.

STRAIN	NUMBER OF X CHROMOSOMES TESTED	NUMBER OF MALE P_1	SEX-LINKED LETHAL MUTATIONS	FREQUENCY OF LETHALS (%)	NUMBER OF MALE P_1 GIVING LETHALS	NUMBER OF F^2 EXAMINED (ESTIMATED)	VISIBLE P_1 MUTATIONS	VISIBLE F_1 MUTATIONS
y Hw F_1 2	849	28	0	0.0	0	8,490	0	2
Or F_7 2	916	31	3	0.33±0.19	3	9,130	1	2+1?

some, resulting in contorted, "hooked" bristles, and a third male was heterozygous for a new third chromosomal recessive, resulting in wings with very scalloped edges. Since the *y Hw* F_3 2 strain was carried by small mass matings, resulting in rapid elimination or homozygosity of new factors, these two mutations had probably arisen at a relatively recent date. In the light of experiments 1 and 2 the results with this strain can most reasonably be explained in terms of the recent loss from the strain of the dominant factor thought to be responsible for the phenomenon.

The Or F_7 2 line yielded three lethals and one visible, a sex-linked recessive characterized by weak, uneven-surfaced wings and rotated genitalia, in a total of 916 X chromosomes. Among the approximately 9130 F_2 males inspected were three single mutants, a Minute due to an unlocalized autosomal dominant, a vermilion-2 type eye, and a vermilion-1 type eye (both 1–33.0). The latter might well have arisen by double crossing over involving the *ClB* inversion, since there was no check on such an event in this experiment. The same possibility does not hold for the vermilion-2, since it is phenotypically distinct from the vermilion carried within the inversion. This rate of mutation, although possibly high, was not thought sufficiently greater than normal to warrant further investigation.

THE NON-RANDOM DISTRIBUTION OF MUTANTS IN THE PROGENY OF CERTAIN FLIES

In previous sections, several cases of a possible non-random distribution of mutations in the offspring of certain flies have been described. A more significant example than any of these is the following: In the F_2 of a cross of a male with rough eyes and missing bristles, found in the second generation of the study, to normal females, the ratio indicative of a sex-linked mutation was secured. In establishing a pure stock of this, mutant males were crossed with their phenotypically normal sisters. Among the 384 offspring of this cross there appeared, in addition to the expected types, two new phenotypes, both represented by a single fly. The first was a male characterized by small, blistered wings and contorted, gnarled bristles, and was subsequently shown to be the result of a recessive mutation in the third

chromosome. The second was a female characterized by a deep, dull red eye. Crossed with her normal-eyed brothers, she produced 149 normal-eyed offspring and 32 with the aberrant eye color, as well as one female with an extreme type of plexus wings. The latter was sterile. Subsequent analysis showed that the eye color was due to a third chromosomal recessive, which later cropped out in several other lines. Males and females showing the new eye color, bred together to establish a homozygous stock, yielded in the next generation ca. 150 flies showing the trait in question, and in addition a yellow-1 mutant and a lozenge mutant (1–27.0).

Several more less striking cases of this nature could be added. But while the data are suggestive of a non-random distribution, they are by no means critical. In an extensive series of observations like the present a clumping of mutations is occasionally to be expected on the basis of chance alone. However, such a clumping is also exactly what would be expected if the factor responsible for the increased mutation rate was a dominant carried by a relatively few flies. No outbursts of mutation similar to the cases briefly described by GOLDSCHMIDT (1937), involving not only the simultaneous appearance in a strain of as many as four mutations but also the simultaneous disappearance of recessive markers, were observed.

THE LOCALIZATION OF THE MUTATIONS

One question which arises in the course of an investigation such as this concerns the distribution of the mutations. Are they scattered at random throughout the chromosomes, or is there a specific effect on certain regions? This question is most conveniently discussed in connection with the 42 new sex-linked factors which were analyzed to a point where they could be assigned a definite locus. These factors were as follows (the first figure in parentheses gives the number of times the mutation occurred; the second, the location): yellow (18) (0.0), multiple (1) (0.0), rough (1) (0.5), prune (1) (0.8), Notch (2) (3.0), carmine (1) (18.9), tiny bristle (1) (19.5), cut (2) (20.0), singed (3) (21.0), scruff (1) (22.0), lozenge (2) (27.0), vermilion (4) (33.0), oblique (1) (37.2), forked (2) (56.7), and bobbed (2) (66.0).

With three possible exceptions they appear to be distributed at random. These exceptions are (1) a high frequency of yellow mutations, also noted by DEMEREC (1937) and TINIAKOV (1939) in their work, (2) the absence of detected mutation at the white locus, although this is thought to be one of the most frequently mutating loci of Drosophila, and (3) the apparent grouping of mutations at about map position 20. Four of the yellow mutations were of the yellow-1 type, and 14 the yellow-2 variety. Except for the yellows, the concentration of mutations at the genetic tip of the X is consistent with the known normal distribution of Drosophola mutations.

DISCUSSION

The methods used in this study to detect visible mutations are undoubtedly of different relative efficiencies. There can be little doubt that the *ClB* method is the most effective when applied to certain types of visible P_1 mutations, that inbreeding of a strain is next in effectiveness, and that the attached-X method and the examination of the F_2 of a *ClB* cross for single mutants appearing as a result of mutation in the F_1 are relatively inefficient procedures. If, notwithstanding, we bring together all the results obtained by any method, we find that a total of 80–84 mutants were detected anong 189,827 flies, exclusive of the controls and exclusive of mutants picked up when exact quantitative records were not being kept. This corresponds to a rate of 43.2 per 100,000 flies and is 1.73 times the normal estimated rate of 25 per 100,000 flies. The total number of lethal mutations detected in the 7565 X chromosomes analyzed was 24–25 which represents a rate of 0.32 ± 0.06 percent. This is 1.8 times the normal rate of 0.18 ± 0.03 as summarized by SCHULTZ (1936). These represent the minimum possible estimates of the increase, since they are undoubtedly based in part on flies in which the factor responsible for the increase was not represented. If we exclude the results with Or F_8 1 (table 2), *y Hw* F_3 2 (table 7), and the balanced b11 line, on the grounds that the rate in these cases appears entirely normal or even, owing to the methods of detection, subnormal, the rate of visible mutation is 47.5 per 100,000 flies, and the rate of lethals, 0.43 ± 0.09 percent. This corresponds to an approximate twofold increase in the frequency of both lethals and visibles. This is still a minimum estimate, since an unknown fraction of the flies within these groups carried the factor responsible for the increase. Thus, in experiments 1 and 2 (tables 3 and 5), out of a total of 23 males tested from a strain showing an appreciably high mutation rate, not more than four or five showed evidence of throwing mutations in unusually high numbers.

The behavior of strains established from three of these flies, and carrying various combinations of their chromosomes, as summarized in tables 4 and 6, indicates that a dominant influence associated with the third chromosome of these males, or perhaps a combination of the first and third, is at the basis of the phenomenon. The combined data of the two tables show that in 18,012 flies whose parents carried a first and second chromosome derived from some one of the three "mutating" males, only two mutations were found, while in 20,729 offspring of parents which either certainly or possibly carried a third from these males, as well as a first and/or second, there were 13 mutations. The difference between these two series, as measured by χ^2, with Yates's correction for continuity, is highly significant. The difficulty of detection of mutations in these balanced strains

is such as to suggest that the rate observed in the second group (62.8 per 100,000) is actually a considerable underestimate of the true rate. Moreover, since the factor is behaving as a dominant and since only a relatively few males appear to carry it, the founder males were probably heterozygous, and in that case only half of the stocks balanced for the third chromosome might be expected to carry the factor. A wild type strain heterozygous for the factor should therefore mutate at approximately four to five times the normal rate.

In the analysis of the results obtained with the balanced lines, the assumption is implicit that the various chromosomes introduced as balancers, or carrying marker genes, themselves had no effect on the mutation rate. There is no reason to doubt the validity of this assumption.

The above interpretation encounters one signal difficulty. How can the significant decrease in the frequency of lethal mutation in the *y Hw* F_3 6 line, from 1.01 ± 0.28 percent to 0.19 ± 0.08 percent in three generations, be explained? If the increase was due to a dominant factor which was certainly not homozygous in the strain and which may be unstable, chance alone might be sufficient to account for the difference between the results of the two tests. Even so, if males 12 and 13 carried a factor increasing the mutation rate by a factor of four, then among the 910 X chromosomes from these two males tested for lethals, there should have been six, instead of none. This point remains the chief complication in the present interpretation of the results.

The estimate of the normal frequency of detection of visible mutation adopted in this work is so much higher than the generally published data that it is certain to elicit doubt. Only further independent work by competent investigators can settle this question. If the lower estimates of the frequency of visibles be accepted, then in the case here analyzed we are forced to assume a considerable increase in the frequency of visibles without a corresponding increase in the frequency of lethals.

GOLDSCHMIDT (1939) has vigorously espoused the role of chromosomal rearrangements, as leading to further rearrangements which are detected through the mutant phenotypes they produce, in such periods of high mutation rate. Both VALADARES (1937) and GOLDSCHMIDT (1939) report the presence of an inversion within their strains. However, it must be emphasized that inversions are sufficiently widespread in Drosophila stocks that any such claims to be accepted must be accompanied by a rigorous demonstration of a causal relationship between rearrangement and mutation, and such a demonstration is thus far lacking. MISS JEAN LANE, of the Department of Genetics, CARNEGIE INSTITUTION OF WASHINGTON, very kindly examined the salivary gland cell chromosomes of the *y Hw* F_3 6 line shortly after the termination of the experiment summarized

in table 2 and was able to detect no gross aberration. The only possible departure from normal observed was a slightly exaggerated tendency toward asynapsis. Moreover, in none of the linkage experiments localizing the various mutations encountered was any evidence of rearrangement found. DR. E. SUTTON found one of the Notch mutations to be characterized by the loss of the 3C7 band from salivary gland cell chromosomes but was unable to detect any abnormality in the chromosomes of the five other sex-linked mutations which she kindly analyzed.

The factor responsible for the present outburst is obviously different from that found by DEMEREC (1937). There are therefore at least two factors, one associated with the second chromosome and the other with the third, which exert a profound effect upon mutation rate in Drosophila. Such factors as these may without exaggeration be regarded as pacemakers of evolution, since by controlling the mutation rate they indirectly control the maximum rate at which a species may change.

As GOLDSCHMIDT (1937) has pointed out, the usual design of Drosophila work militates strongly against detecting such cases of high mutation rate. Further investigation may show that a very considerable portion of the mutation occurring in nature comes in such spurts, resulting in the formation of localized populations of relatively high genetic heterogeneity. Some evidence of this has already been obtained by SPENCER (1942). Should this be the case, then here is a mechanism which may rank in importance with geographical isolation in the process of species differentiation, and one which, in any event, offers a valuable supplement to isolation.

SUMMARY

A case of high mutation frequency in an inbred Oregon wild type line of *Drosophila melanogaster* is described and analyzed. From data based on approximately 208,000 control and experimental flies it is concluded that:

(a) The normal rate of detection of new mutants is about 25 per 100,000 flies under conditions of moderate inbreeding.
(b) In this particular case there was a two to fourfold increase in the frequency of occurrence of sex-linked lethal and sex-linked and autosomal visible mutation.
(c) A dominant influence of the third chromosome, or, less probably, of a combination of the first and third chromosomes, was responsible for the increase.

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REVERSION FROM ROUGHEST TO WILD TYPE IN DROSOPHILA MELANOGASTER

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ROUGHEST is a sex-linked, recessive character of *Drosophila melanogaster* manifested phenotypically in bulging, roughened eyes that have facets irregular in size and shape. The allele roughest^3 (*rst*^{3}) was obtained by GRÜNEBERG (1935) from an X-ray experiment. Eyes of *rst*^{3} flies show mosaics of smooth and rough facets with rough patches more pronounced in the males than in the females. Roughness is accentuated if flies are raised at temperatures between 16 and 19°C; at higher temperatures the females more frequently overlap the wild type in appearance. Viability and fertility are good in both sexes. Genetic analysis (GRÜNEBERG 1935) indicated that *rst*^{3} is associated with a long inversion in the X chromosome (In(1)*rst*^{3}), extending from the roughest locus to the right of the gene bobbed. Preliminary cytological studies by P. CH. KOLLER (reported in GRÜNEBERG 1935) showed that the loop present in the salivary gland chromosomes of flies heterozygous for the inversion extends from a point between the loci of facet and ruby to the proximal heterochromatic region of the X. Subsequent analysis of salivary gland chromosomes by EMMENS (1937) disclosed that the left break of the inversion is in the 3C2 to 3C4 region. Studies made in this laboratory have confirmed EMMENS's interpretation; the left break follows band 3C3 (BRIDGES's 1938 map) and the inversion extends to about subdivision 20B. It does not include the nucleolus-organizing region, as can be seen best in prophases of dividing neuroblasts (fig. 1). The 3C4 band is thus associated with the roughest locus (confirmed by DEMEREC and SLIZYNSKA 1937), which on the genetic map is placed at 1.7 (GRÜNEBERG 1937, from an analysis of *rst*^{2}, which behaves as an allele).

Reversion of In*rst*^{3} to wild type was reported by GRÜNEBERG (1936, 1937). The change occurred spontaneously in a stock homozygous for the *rst*^{2} inversion which also carried the genes yellow, carnation, and bobbed. Since these recessive markers persisted in the reverted material, GRÜNEBERG felt that the possibility of contamination was excluded; this is of course the most plausible explanation. It would be possible, however, to secure a gamete containing yellow, carnation, and bobbed, but lacking the roughest inversion, by a coincidence of rare events, including insemination of an In*rst*^{3} female by a wild type male and production of a triple crossover between the chromosomes of a heterozygous daughter. Since such triple crossovers should be extremely rare, it would be expected that

any culture in which a chromosome of this type was perpetuated would also contain numerous wild type flies, unless the further assumption were made that the progeny of the heterozygous female consisted of this one triple crossover individual. GRÜNEBERG's statement (1937, p. 170) suggests that all the flies carried *y*, *car*, and *bb*. His genetic analysis of the reversion indicated that the wild type sequence of genes had been restored; and supplementary studies of salivary gland chromosomes by EMMENS (1937) confirmed this interpretation, although it must be emphasized that the determination of identical breakage points in heterochromatic regions can be made only with some degree of uncertainty (*cf.* GRIFFEN and STONE 1940). Inasmuch as complete reinversion on a purely chance basis seemed extremely unlikely, GRÜNEBERG suggested "that a pre-existent configuration within the X chromosome has probably determined the points of breakage which led both to the original inversion and to the reinversion." The experiments here recorded were designed to measure the dependence of the reversion phenomenon on reinversion.

Reversion of rst³

Roughest males from the stock In(1)*rst³*, *rst³ car bb* were treated with 4000 roentgens of X-rays and mated with attached-X females (*yy*) in order that reversion might be detected in the F_1 males. It was thought that the more pronounced expression of the roughest character in flies of this sex would permit most readily the detection of any deviation in phenotype toward the wild type. This anticipation was realized to a surprisingly high degree, and the first experiments yielded 22 males having *car* eyes in which the pattern of facets was as regular and uniform as in the wild type. None of these 22 males produced any offspring although the females with which they were placed deposited some eggs in the cultures.

It was necessary, therefore, to revise the procedure so that the altered X chromosome might be carried in the heterozygous rather than in the hemizygous condition, and this was accomplished by mating the irradiated males with In*rst³* females. In order to facilitate detection of reversions, the flies were raised at a temperature of about 19°C.

In these experiments a total of 21,104 F_1 females were examined. Among them were 171 that appeared phenotypically non-roughest; the remaining 20,933 showed the roughest character. Of the 171 that were apparently non-roughest, 72 were selected at random for analysis. Twenty-five of these produced no offspring; seven had low viability and were lost or discarded; 23 simulated wild type owing to the variability of expression of *rst³*; the remaining 17 represented true reversions.

Assuming that the proportions found in this sample are characteristic of the whole population, calculations can be made concerning the approxi-

mate frequency of reversion at 4000 roentgens. Some consideration must be given to the proportion of true reversions among the individuals of low viability and those that proved sterile. Probably the amount of simulated reversion is no greater in the group showing low viability than among the tested flies—namely, about one-third of the total. A check was made of the proportion of sterility among F_1 roughest females (not showing reversion) whose fathers had been exposed to 4000 roentgens of X-radiation Of 200 such females that deposited eggs after mating with In*rst*^{3} males, 24 failed to produce any offspring. From these tests it may be inferred that sterility which affects more than 12 percent of females carrying the irradiated chromosomes is to be attributed to chromosomal changes associated with the reversion process. On the basis of these estimates it has been calculated that about 90 of the 171 phenotypically wild type females represented true reversions of *rst*^{3}, or that reversion was occurring in about 0.4 percent of all gametes tested. Exact reinversion would not be expected to occur so frequently. Accordingly, cytological studies were undertaken to determine the precise nature of the changes induced in 17 of the reversions.

Salivary gland chromosome analysis of reversions

The reversions, except for the two that were viable when homozygous, were maintained in stock by balancing with In dl-49, *y Hw m*^{2} *g*^{4}. Salivary gland chromosomes for cytological studies were secured either from larvae from these cultures or from matings between virgin females from the stocks carrying reversions and In*rst*^{3} or *rst*^{2} males.

One of the seventeen reversions associated with a change restricted to the X chromosome was lost, presumably by crossing over, before analysis could be completed. The remaining sixteen showed rearrangements of various types, as reported in abstract form in GENETICS (KAUFMAN 1940) and in Year Books No. 38 and No. 39 of the CARNEGIE INSTITUTION OF WASHINGTON (1939, 1940). All sixteen involved breaks in the proximal heterochromatic region of the In*rst*^{3} X chromosome. Four of the changes were inversions, and in two others there was a combination of inversion with translocation. There were two cases involving transfer of the nucleolus-organizing region from its usual position in the X between 20B and C to an autosome. Seven of the rearrangements were reciprocal translocations; in one of these an additional independent translocation was present. Detailed information concerning the breakage points incorporated in each rearrangement is furnished in table 1.

The pattern of recombination revealed in the salivary gland chromosomes is described in table 1 under the caption "Type of rearrangement." Since two of the seventeen rearrangements studied showed translocation of the nucleolus-organizing region, it is suggested that the method here

TABLE 1

EXPERIMENT NO.	TYPE OF REARRANGEMENT	POSITIONS OF BREAKS INDUCED IN THE In(1)*rst*^{3} CHROMOSOME
319-51-A	Inversion in X	In proximal heterochromatin, probably in 20B adjacent to 3C4; other break between 7A5 and 7A6.
319-55-K	Inversion in X	One break in heterochromatin as in preceding; other between 18F3 and 18F5.
319-22-7	Inversion in X	In proximal heterochromatin as in preceding; other break between 19E2 and 19E3.
319-52-A	Inversion in X	Heterochromatic break as in preceding; other break between 9A3 and 9A4.
319-41-A	Inversion in X combined with reciprocal translocation	Inversion extends from proximal heterochromatin to *c.* 9C5 or 9C6; translocation with 51E7 break in 2R.
319-24-21	Same as preceding	Inversion extends from proximal heterochromatin to about 5C9 or 5C10; translocation with 87F break in 3R.
319-1-C	Transfer of nucleolus-organizing region from the X chromosome to 3L	Two breaks in the proximal heterochromatin of X, probably one proximal to 20C and one distal to 20B so as to include nucleolus-organizing region which was removed and inserted in 3L at 72E. Photograph of nucleolus in 3L shown as figure 2.
319-22-25	Similar to preceding except n.o.r. is inserted in 2L	Heterochromatic breaks in X as in preceding, n.o.r. inserted in 2L at 27E.
319-18-1	Reciprocal translocation	One break in proximal heterochromatin of X, probably in 20B adjacent to 3C4 but distal to n.o.r.; other break in 26B of 2L.
319-1-D	Reciprocal translocation	X break as in preceding; other break in 23F of 2L.
319-40-1	Reciprocal translocation	X break as in preceding; other break in 58D of 2R.
319-43-C	Reciprocal translocation	X break as in preceding; other break in 93E of 3R.
319-18-2	Reciprocal translocation	X break as in preceding; other break in 84F of 3R.
319-22-23	Reciprocal translocation	X break proximal to the n.o.r.; other break in 3R at 93B. Pattern of recombination as follows: Tip X . . 3C3/20B . . . 3C4/20B . . . n.o.r. . . . /93B . . . centromere 3R. Tip 3R . . . 93B/20C or D . . . centromere X.
319-23-5	Reciprocal translocations X/3L and X/3R	One break in X to left of n.o.r. translocated with 65C in 3L; another break in 11A of X translocated with 99F in 3R. Pattern of recombination: Tip 3L . . . 65C/ca20B . . . centromere of X. Tip X . . . 3C3/ca20B . . . 11A/99F . . . centromere of 3R·3L . . . 65C/ca3C4 . . . 11A/99F . . . tip of 3R. It would seem that the 11A/99F exchange is quite independent of the change associated with reversion.
319-20-9	Mutual exchange involving X, 2R and 3R	X break in heterochromatin as in preceding; 2R break in chromocenter; 3R break in 89E. Pattern at recombination appears to be X tip on 3R base, 2R tip on X base, 3R tip on 2R base.

described may offer a satisfactory procedure for securing such changes when desired for experimental purposes.

Although one of the breaks incident to each rearrangement occurred in the proximal heterochromatic region of the In*rst*^{3} X chromosome and most frequently in 20B close to the 3C4 band, the precise location could not be determined cytologically. Two factors contributed to the uncertainty: first, the 3C4 band is rarely discernible when lying in contact with heterochromatin, either in the In*rst*^{3} chromosome or in the derived rearrangement; second, the accurate location by salivary gland chromosome analysis of a break in the proximal heterochromatin of the X is complicated by the diffuse nature of the banding in this region and the irregular pairing

that occurs with proximal heterochromatin of other chromosomes. One guide toward more accurate localization provided by the X chromosome is the position of the nucleolus-organizing region. In the wild type chromosome the nucleolus is organized in the region adjoining 20B and C and lying close to the centromere (KAUFMANN 1939). In the In*rst*^{3} chromosome the nucleolus occupies the same relative position, indicating that the inversion does not encompass the nucleolus-organizing region (fig. 1). The

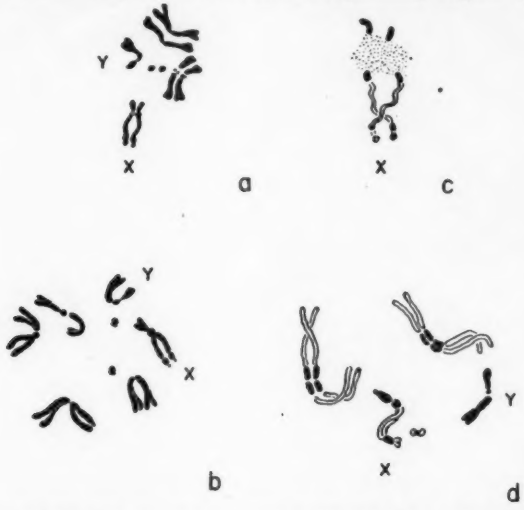

FIGURE 1.—Mitotic chromosomes from ganglion cells of *rst*^{3} flies. a, b, d from males; c from female. All show X chromosome constrictions. In prophases c and d heteropyknotic regions are represented in solid black, remainder of chromosome in outline. Nucleolus is stippled. Note that *rst*^{3} inversion has the proximal break in that part of the heterochromatic region which in the wild type X lies distal to the nucleolus.

nucleolus remains close to the centromere in all but three (319-1-C, 319-22-25, and 319-22-23) of the X chromosomes derived from flies that had undergone reversion from roughest to wild type. Accordingly, any break occurring between the nucleolus-organizing region and the 3C4 band is restricted to subdivision 20B or its line of separation from 3C4. But it could not be determined cytologically whether or not more than a single line of fracture occurred in this region in producing the different rearrangements analyzed.

As was stated earlier, all of those reversions of *rst*^{3} that had been secured as F_1 males proved to be sterile. Among the seventeen reversions chosen for analysis, fifteen likewise produced only sterile males. Inversions 319-22-7 and 319-52-A, however, produced fertile males and therefore could be maintained as homozygotes. They were carried as stocks in this way both at 19 and at 25°C for nearly two years, during which time inspection of the

flies when transferred to new bottles showed that they had the smooth wild type eyes characteristic of reverted roughest[3]. In the early part of 1941 a few roughest flies were found in these two stocks. Salivary gland chromosomes obtained from the larval descendants of flies having rough eyes showed that no modification of the sequence of banding had occurred subsequent to the change associated with the reversion of *rst*[3]. It seems,

FIGURE 2.—Nucleolus-organizing region in left limb of third chromosome as a result of translocation accompanying reversion 319-1-C. The inversion loop is caused by pairing between chromosomes heterozygous for Inversion (3L) Payne. The Payne inversion was present in the chromosome that received the nucleolus organizer.

therefore, that the original suppression of *rst*[3] in these two cases may have been dependent not only on the primary change but also on the presence of a system of modifiers. Subsequent alteration of this system would permit the expression of the roughest character in those individuals in which the suppressors were no longer effective and would be responsible for the *rst* flies which then appeared in the stocks.

DISCUSSION

The problem of differential fragility

The present study has shown that phenotypic reversions of In*rst*[3] may be brought about by chromosomal alterations other than reinversion. GRÜNEBERG suggested that both the original production of the inversion by irradiation and its subsequent reinversion may have involved exchanges at the point of overlap in chromosome loops formed because of attraction

between regions that were identical or at least similar in origin, the one representing a repetition of the other. To secure the *rst*^{3} inversion and reinversion on such a predetermined pattern, 3C and 20B should contain homologous sections. No relationship between these subdivisions can be recognized on the basis of similarity of gene content, since 20B is essentially inert, whereas several bands of subdivision 3C have been correlated with known genes: 3C2 (or 3C3) with white, 3C4 with roughest, 3C7 with facet. However, if 3C should encompass some inert material similar to that of subdivision 20, as PROKOFYEVA-BELGOVSKAYA (1939a, 1939b) first inferred from observations of pairing between these two regions in salivary gland chromosomes, there would be a structural basis favoring loop formation, at least in salivary gland chromosomes. It does not necessarily follow, even if loop configurations were demonstrable in sperm nuclei, that this type of intrachromosomal conjugation is prerequisite to the production of new rearrangements. Although propinquity between strands is essential in order that broken ends may form new combinations, such contact may not exist at the time the positions of the breaks are determined. Some evidence in this connection has been presented by MULLER (1940) and KAUFMANN (1941a) who found that potential breaks induced by X-rays in sperms of *D. melanogaster* apparently do not participate in the formation of rearrangements until after the sperm nucleus has entered the egg.

If the regions delimiting the *rst*^{3} inversion were more susceptible than adjoining parts of the chromosome to breakage per unit of mitotic chromosome length, either as a result of previous breakage or because of the type of chromatin involved, reinversion should occur with great frequency. Using phenotypic reversion as a basis for selection, the position of one break in any chromosomal rearrangement is localized, apparently requiring an alteration in the relationship of the 3C4 locus to the proximal heterochromatic materials of the X. It may be estimated, on the basis of former studies of break frequency in heterochromatin of the X at 4000 roentgens, that about one out of every nine breaks in this region is disclosed by the reversion of *rst*^{3}. Probably the method is much more sensitive than this estimate suggests, since the cytological analysis indicated that not all breaks in heterochromatin are detected, but more particularly those in 20B distal to the nucleolus. The positions of the other breaks involved in detectable rearrangements appear to be random, either in the X or in one of the autosomes. This evidence suggests that any reinversion that might be induced by irradiation would depend on the chance distribution of breaks, rather than on any underlying structural pattern involving contact between regions of preferred breakage. The occurrence of an analogous situation in the production of a spontaneous rearrangement can be only surmised.

Thus, the rough[3] inversion, as well as the delta-49 inversion previously tested in this laboratory (KAUFMANN 1939), has not been found to show increased fragility to X-rays in the regions at the limits of the inverted sections. At variance with this finding are such reports as those of SITKO (1938a, 1938b), presenting evidence that the regions of new unions established as a result of inversion and translocation are more susceptible to induced breakage than such regions in the normal chromosomes; the somewhat similar evidence adduced by TZUBINA (1939) from the incidence of changes affecting the expression of cubitus interruptus, presumably through the production of translocations in a fourth chromosome which previously had been broken and reattached in translocation; and the data provided by GRIFFEN and STONE (1940) indicating that the attachment between the white locus in the X and its fourth chromosome associate in the w^{m5} translocation represents a weak spot. It is conceivable that regions adjacent to breakage points in different rearrangements may behave differently. It is also possible that some of the conflicting data may be reconcilable on the basis of differences in recombination rates of the various displaced sections and are therefore not directly attributable to differential fragility. The writer (KAUFMANN 1941b) has maintained that the cytological analysis of salivary gland chromosomes provides the best method for measuring the distribution of all breaks involved in these rearrangements. Additional experiments with other inversions are being conducted along these lines.

Reversions and reverse mutations

Since reversion of rough[3] in each of the cases tested involved some perceptible chromosome rearrangement, an effort was made to determine whether the phenomenon of reverse mutation might require similar changes. Several loci were selected, including forked in the X chromosome and peach in the third. These two had shown in earlier studies (summary by TIMOFEEFF-RESSOVSKY 1937, p. 89) a reverse mutation rate equal to or greater than that of direct mutation. Flies carrying these and other markers were irradiated with about 4000 roentgens of X-rays and their offspring examined for reversions. With the assistance of MR. TAYLOR HINTON, MR. FLOYD BLIVEN, and MISS JEAN LANE, 25,565 flies were examined for changes at the forked locus, with entirely negative results. No changes to wild type were found among 11,900 flies surveyed for changes in peach. GRIFFEN and STONE (1939), however, have reported briefly that the spontaneous mutations apricot, miniature, and forked were caused to revert to normal alleles by irradiation and that the single case of each studied genetically and cytologically revealed no detectable gene rearrangement.

Failure to secure reversions of point mutations in the experiments summarized above prompted a further effort to secure changes in a mutation associated with a chromosomal rearrangement. For this purpose white-eyed males of the stock 258-52 (DEMEREC), which carry an inversion in the X chromosome extending from 3C to 8E, were treated with 3000 roentgens of X-rays. Among the first 12,000 gametes that we tested there was no change from white toward the wild type. Curly and Glazed, which accompany inversions in the second chromosome, have been found to revert to normal with changes in chromosome associations (SUCHE, PARKER, BISHOP, and GRIFFEN 1939, GRIFFEN and STONE 1940); and the Bar^S rearrangement (T(1; 4)B^S) has been reported by GRIFFEN (1941) to give occasional complete returns to normal eye shape. Reversions of genes producing mottling will be considered in the following section.

Roughest[3] as a position effect

The number of distorted facets in the eyes of roughest flies may vary with the sex of the individual, the temperature at which the cultures are raised, or the genetic environment afforded by neighboring genes (DEMEREC and SLIZYNSKA 1937). Roughest thus belongs to that group of characters which are expressed in mottling or mosaicism of somatic tissues. As SCHULTZ (1936) and others have emphasized, mottling may be induced by the proximity of a gene to heterochromatin; and *rst*[3] is of this type. The conditions necessary for the production of irregular eye facets are met by the properties of the proximal region of the X chromosome. Transfer of the 3C4 locus to this region induces roughest; it is a position effect. Removal of the gene from this environment may prevent the mottling. It is not necessary that the original gene sequence be reestablished in order to ensure reversion, as GRÜNEBERG'S experiments suggested; although completion of the cycle of inversion and reinversion, with the appearance and disappearance of the character, would provide the most crucial proof of the existence of a position effect. There are many situations in the chromosome that provide environments, as far as the expression of roughest is concerned, parallel to that of the gene in its normal position. The experiments reported in this paper shed some light on the factors involved.

In the first place it is clearly shown that the appearance and disappearance of the roughest effect is dependent not merely on proximity to heterochromatin or removal therefrom. As was mentioned previously, subdivision 3C probably contains some heterochromatic materials, so that the 3C4 locus in the wild type chromosome may not be situated in a totally euchromatic environment. However, the quantity of heterochromatin incorporated in 3C is probably small, since the break frequency data (KAUFMANN 1939) suggest that this subdivision is not disproportionately longer in the

mitotic chromosome than are adjoining euchromatic sections. Three of the reversions of roughest indicated, moreover, that mottling is not always evoked when the 3C4 locus lies adjacent to proximal heterochromatin. In none of these three had the roughest inversion been altered perceptibly, but the proximal heterochromatic relationships had been disturbed in two cases by removal of the nucleolus and some adjoining materials from the chromosome, and in the third case the entire distal portion of the X, including the inversion and the nucleolus-organizing region, had been translocated with the limb of another chromosome. From these considerations it would appear that the production of variegation in In*rst*^{3} requires the proximity of the 3C4 band to heterochromatin, but that this heterochromatic material must be located proximally in the chromosome, and not in some intercalary region. There is the further suggestion that proximity to the nucleolus, or the nucleolus-organizing region, is essential. The alternative possibility cannot be overlooked that the effect depends on the quantity of heterochromatin present in the chromosome, as PANSHIN (1938) suggested in explanation of the type of variegation observed for white mottled[11]. In the *D. melanogaster* chromosome heterochromatin is most abundant adjacent to the centromere. Removal from this region of sufficient material to be detected as the translocation of the nucleolus might appreciably reduce the effective quantity remaining. A significant check of the dependence of variegation on proximity to the nucleolus would be the production of reversion from *rst*^{3} following transfer of the 3C4 locus to the proximal heterochromatic region of the limb of one of the autosomes. So far no reversion of this type has been discovered. On the other hand,—apart from the cases here reported,—there are some further indications that the region of the nucleolus may be of some importance in influencing the variegation process. In a report to the 1939 International Genetics Congress, DEMEREC (1941) noted that certain parts of the heterochromatin are more effective than others in producing mottling of genes located in subdivision 3C. In two X chromosome inversions, in which one break had occurred in 3C and the other to the right of the nucleolus-organizing region, so that the nucleolus occupied a position near the tip of the chromosome, there was no mottling in any of the loci tested. In two other inversions, in which the break was to the left of the nucleolus-organizing region, mottling was induced in the loci brought near to the nucleolus. The amount of heterochromatin remaining adjacent to the centromere varied in these different cases; but quantity does not seem to be the factor controlling variegation, for the reason that mottling of the loci concerned is induced by the proximal heterochromatic regions of all the chromosomes, including the fourth. Other studies showing qualitative differences in heterochromatic materials have been summarized by V. V. KHVOSTOVA

in Drosophila Information Service **15**, pp. 25 and 26, 1941 (see also PANSHIN 1938). In these extensive investigations of rearrangements affecting the expression of the fourth chromosome gene, cubitus interruptus, it was found that the proximal parts of the "inert" regions of the X and Y chromosomes are analogous to the "inert" regions of the autosomes in their failure to suppress the dominance of the wild type allele; whereas the distal regions of the Y and the part of the "inert" region of the X to the left of bobbed are analogous to the "active" parts in weakening the dominance of ci^+. Additional studies of changes in roughest^3 induced in stocks containing displaced nucleolus-organizing regions, or extra Y chromosomes, might provide an answer as to the relative importance of different parts of the "inert" material. If a definite rôle of the nucleolus or its organizer in the variegation process could be established, further evidence would be available concerning behavior of specific genes in relation to the nucleoprotein metabolism of the cell (see review by SCHULTZ 1941).

One recent report (GRIFFEN and STONE 1940) has disclaimed the importance of heterochromatin in inducing variegation of a white mottled (w^{m5}) and its derivatives. The argument is two-pronged: first, that the w^{m5} rearrangement (the tip of X down to 3C2 translocated to the fourth chromosome) did not involve heterochromatin, and second, that the mottling persisted after the 3C2 locus had been transferred to various intercalary euchromatic loci. The first of these premises is open to the serious criticism that numerous observations of the fourth chromosome, including its behavior in pairing in the salivary gland nuclei, have indicated the existence of proximal and probably also terminal heterochromatin (BRIDGES 1935; SCHULTZ 1936; PROKOFYEVA-BELGOVSKAYA 1937; BAUER, DEMEREC, and KAUFMANN 1938; and others). Conceding the presence of heterochromatin in the fourth chromosome, the transfer of 3C2 to new locations may have been accompanied by some of this "inert" material. Assuming, however, the absence of such substances in contact with 3C2, there remains the possibility that in this particular change the gene is extremely sensitive to influences of heterochromatin effective through comparatively long distances cytologically. It has already been established that heterochromatic regions are distributed along the X chromosomes (PROKOFYEVA-BELGOVSKAYA 1939b, PROKOFYEVA-BELGOVSKAYA and KHVOSTOVA 1939, KAUFMANN 1939), and there is good reason to suspect their existence in the autosomes. GRIFFEN and STONE described one rearrangement in which 3C2 in contact with 3F2 permits pronounced mottling. By all available criteria, 3F2 is euchromatic, but it is not far removed from the heterochromatic zone of 3C nor from other similar regions in division 4. The expression of a position effect under the influence of some distant alteration in the chromosome has been discovered both for the stable changes and for variega-

tion at various loci, including hairy, curled, Bar, roughest^3, white, and other loci of the Notch region. An analysis by FANO (DEMEREC, KAUFMANN, SUTTON, and FANO 1941) of genetic changes at the Notch locus indicates that about one-tenth of all breaks occurring sufficiently close to the locus affect its phenotypical expression. It is not necessary that the break be adjacent to the changed locus, particularly in transfers to heterochromatin. An influence extending over about 50 bands as measured in the salivary gland chromosome has been observed by DEMEREC (1940). Such remote effects must be appraised fully before the influence of heterochromatin on variegation can be denied.

SUMMARY

Reversion of roughest^3 has been induced in progeny of irradiated *rst*^{3} males. Reversion is associated with various chromosomal alterations, each involving at least one break in the proximal heterochromatic region of the X chromosome adjacent to the position occupied by the roughest locus (3C4 in the salivary gland chromosome) in the *rst*^{3} inversion.

The 17 rearrangements analyzed cytologically showed inversions and translocations, but no reinversion. There were no indications that the positions of the breaks observed in these alterations were determined by preexisting "weak" spots in the chromosomes. In two rearrangements, the nucleolus-organizing region had been removed from the X, so that the roughest locus was brought closer to the centromere. In another, nearly all of the X, including the roughest locus and the adjoining nucleolus-organizing region, had been translocated to an autosome.

This evidence, considered in the light of other similar studies, suggests that the phenotypic expression of roughest^3 as mottling or mosaicism of the eye facets is dependent on the proximity of the roughest locus to that portion of the X chromosome which includes both the nucleolus-organizing region and the centromere. The *rst*^{3} position effect appears to depend therefore not only on quantity of heterochromatin available, but also on qualitative differences within the heterochromatic materials.

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A HEREDITARY VARIEGATION IN TOMATOES[1]

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FOR several years an occasional variegated tomato plant has been observed in our field cultures. The variegation often appears to be due to bud variation, since one part of the plant is variegated while the rest seems to be normal.

The upper surface of a variegated leaflet is composed of normal green and abnormal pale-green portions (fig. 1, C). The pale-green color of the abnormal portions resembles that of the lower surface of a normal leaf, which is decidedly paler than the upper surface. Pale-green leaflets are smoother than normal leaflets and are often rolled upwards. The area of the pale portion is reduced, so that the halves of a leaflet on either side of the midrib may be unequal, or the lamina may be distorted (fig. 1, A).

The distribution of normal and pale-green areas is very irregular. On a variegated shoot, the pale condition may affect a whole leaf, all the leaflets on one side of the rachis, one half of a leaflet at one side of the midrib, or a very small patch of the lamina. The stem is usually marked with alternating normal green and pale-green stripes, which vary in length and in width (fig. 1, B). A stripe may be broad, almost completely enveloping the stem, or it may be very narrow, constituting a mere striation. A stripe may be confined to a single internode, or it may be longer, passing upward into an inflorescence or into another internode. Leaves and stems which at first glance seem to be wholly pale, usually have small areas of normal tissue.

In the field, the leaves of much-variegated shoots look somewhat like those of the gene mutant wilty (MACARTHUR 1934). The leaves of wilty (*wt wt*) and of variegated plants are much less uprolled in the greenhouse; consequently, the spotty appearance of some variegated leaves is more conspicuous there than in the field. The upper surface of the leaves of wilty is normal green.

Purple anthocyanin develops in the stem tips and petioles of plants containing the normal allele *A*1 (MACARTHUR 1931), especially in cool weather and with exposure to direct sunlight. On variegated shoots, when anthocyanin is abundant, there is a deficiency of purple in the pale portions, and purple and nonpurple stripes occur side by side on the young stem. A little brownish-purple pigment, usually limited to thin lines, may occur in the petioles of wholly pale leaflets. The variegation therefore affects both the green (chlorophyll) and the purple (anthocyanin) pig-

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mentation of the plant. The difference in color and in smoothness of leaf surface caused by variegation, is best seen in dwarf (*d*1*d*1) plants which contain the gene *A*1 for purple anthocyanin and have made nearly their

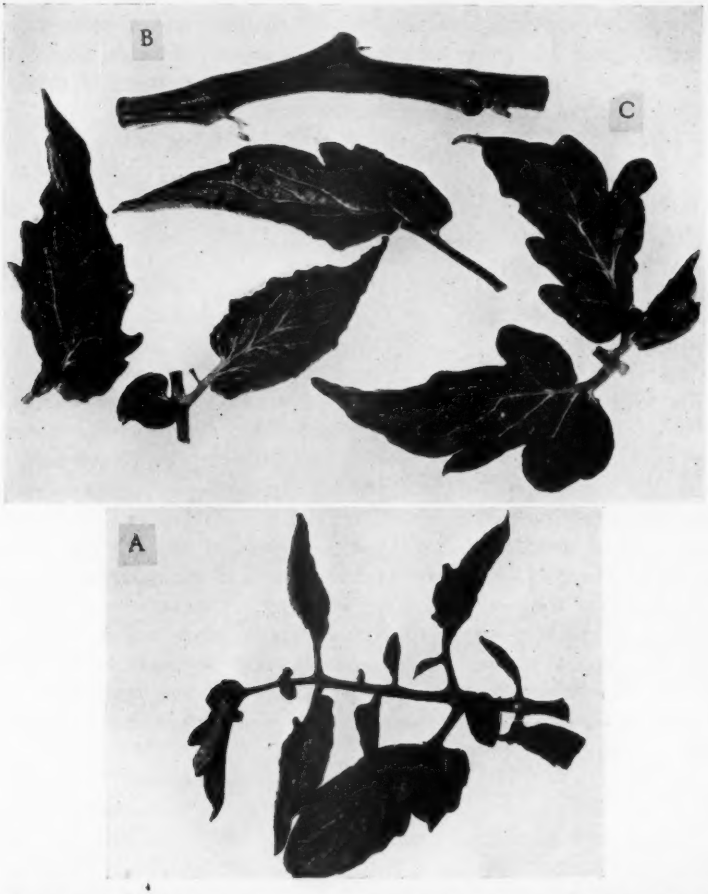


FIGURE 1.—Stem and leaves of a variegated tomato plant. Abnormal tissue is paler than normal tissue. A, leaf: large leaflet below is normal; smaller leaflets are uprolled and mostly composed of abnormal tissue. B, variegated stem. C, leaflets: upper one of pair at right has abnormal patches; two in the center are largely abnormal; leaflet at left is mostly normal.

full vegetative growth and have not been pruned. No variegation has been observed in unripe or ripe fruits.

In one population derived from selfing a much-variegated plant, practically every shoot became much variegated. One plant was much stunted

and distorted, as if affected with cucumber mosaic, and was entirely unfruitful. The others were very unproductive. One of these much-variegated plants was crossed with a nonvariegated male. Two weeks after the seeds resulting from this cross had been planted, variegation was perceptible in the cotyledons and true leaves of the seedlings. In populations from less-variegated parents, variegated shoots appeared only after the plants had reached their maximum size, two or three months after transplanting; the rest of the leaves and shoots remained normal.

A branch which has abnormal pale areas extending over most of the stem and leaves, is, as a rule, unfruitful. On the same plant a normal or slightly variegated branch is, as a rule, fruitful.

CYTOLOGY AND HISTOLOGY

Normal tomato flowers occur on branches having normal leaves and are a bright clear yellow when mature. Flowers on a branch which has abnormal pale-green stems and leaves, remain pale yellow, never open fully, and have pale thin anthers containing little or no pollen.

The pollen from variegated plants is characterized by irregularity in size and by the presence of abnormally large grains, which appear to result from complete failure of division of the pollen mother cell. Such pollen is extremely rare in normal diploids but can be found in nearly every smear from variegated plants. The microspores of the tetrad, instead of forming a tetrahedron, tend to be arranged in one plane. Tetrads from badly affected plants often have unequal microspores, and dyads or pentads seem to occur more frequently than in anthers from normal plants. Except for occasional non-reduction, which also occurs to some extent in normal diploids, meiosis is normal. There are 12 pairs of chromosomes as in normal diploids. The difference in cytological behavior seems to be due to an abnormal condition in the cytoplasm, which prevents cytokinesis or makes it slow or irregular.

In a normal shoot, a layer of palisade cells lies just beneath the epidermis (fig. 2, A). In the developing shoot of a variegated plant, some of these cells fail to undergo normal differentiation and look much like the cells of the spongy parenchyma (fig. 2, B). For this reason, the upper surface of the abnormal portion of a variegated leaf is gray-green and resembles the lower surface in color. Low magnification of the upper surface of the normal portion of a variegated leaf shows a large number of small cell ends so closely massed as to cause buckling and to partially obscure the veins. In the abnormal portion of the leaf, large, irregular, loosely arranged cells can be seen beneath the epidermis. Sections of variegated leaves show that the gray-green areas lack the palisade layer (fig. 2, B). The irregularly shaped cells contain plastids, but since relatively few cells are present in

abnormal regions, much less chlorophyll is present than in normal portions of the leaf. In abnormal regions, starch grains have been observed in the chloroplasts, and the latter appear to be normal in color. In the pale-green area of a variegated leaf, the outer periblem layer of the upper surface is either modified to form spongy parenchyma or is absent.

In normal regions of the stems, two to four layers of chlorenchyma lie immediately below the epidermis. Some of these cells may contain purple

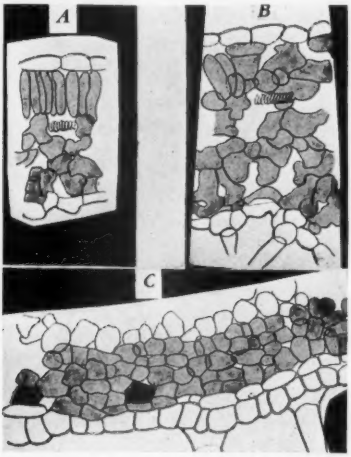


FIGURE 2.—Drawings from freehand sections of fresh tomato leaves and stem. A, normal-green portion of a leaf. B, pale-green portion of a leaf. C, portion of variegated stem including one normal-green and two pale-green stripes; the subepidermal cells in the pale regions lack chlorophyll. ×360.

anthocyanin, especially on the side of the stem exposed to direct sunlight. In the abnormal regions, one or, occasionally, two layers of large flat cells lie just beneath the epidermis. These cells contain no chloroplasts, and neither they nor the two or three layers of chlorenchyma beneath them develop anthocyanin (fig. 2, C). Except for the presence of this colorless subepidermal layer, which gives a gray-green cast to the abnormal stem stripes, the stems are normal. Occasionally, the chlorenchyma from a normal region of the stem tends to slightly overgrow the abnormal subepidermal layer.

TRANSMISSION TESTS

It was at first suspected that the variegation was due to a virus, but its appearance did not correspond to that of any known virus disease. DR. S. P. DOOLITTLE, Senior Pathologist of the UNITED STATES DEPARTMENT

OF AGRICULTURE, made infection tests at Washington, D. C., by rubbing the leaves of healthy tobacco and tomato plants with juice from tissue of variegated tomato plants and by inserting fragments of this tissue into the stems. Ordinary tobacco mosaic was recovered from one variegated plant, but in no case were the peculiar symptoms of variegation transmitted to normal tomato plants. (Leaves of tomato plants affected with tobacco mosaic develop a palisade layer and so differ from those of the variegated tomato.)

Subsequently, at Riverside, variegated scions were successfully grafted onto three normal plants, and normal scions were successfully grafted onto two variegated plants. No symptoms of variegation were found on the shoots of the normal plants and normal scions, even after stock and scion had been growing together for three months.

The nontransmission of the variegation by these means indicates strongly that it is not caused by an ordinary virus. Whole families consisting of many hundreds of plants growing in rows adjacent to variegated plants have remained free from variegation.

GENETICS OF VARIEGATION

Most of the variegated plants can be traced to a simple trisomic plant, triple-H (triplo-IV), C218-8-1, raised in 1932, which has been crossed with various unrelated plants, but a few authentic cases appear to have an independent origin. Variegation was not systematically recorded until September, 1939. The number of variegated plants at that time was probably underestimated, since in some cases variegation was very slight and easily overlooked. In 1940, and especially in 1941, a more careful search was made, so that the records for those years are more complete. For brevity, "V" and "NV" will be used to indicate "variegated" and "nonvariegated," respectively.

Breeding data of V and NV plants are presented in table 1, and the pedigrees of the plants concerned are shown in figures 3, 4, and 5.

A majority of the plants in three small families from selfing parents C373-1, C373-2, and C373-3 (see table 1 and fig. 4), grown in 1939, were variegated. Two families from V F_2 parents C373-2-1 and C373-2-2, contained 25 plants, all of which were variegated. Apparently, V and NV plants segregated out in two other F_3 families from parents C324-2-1 and C324-2-2, V plants again predominating. Two F_1 families, C430 (fig. 3), from V ♀ pollinated by NV? (probably nonvariegated) ♂, and C519 (fig. 3), from V×NV, consisted wholly of V plants. These data suggest that variegation depends on a gene or on genes and is dominant.

In 1940 and 1941, however, two F_2 families from the sibs C430-1 and C430-2, consisting of 43 and 24 plants, respectively, were all variegated.

TABLE 1

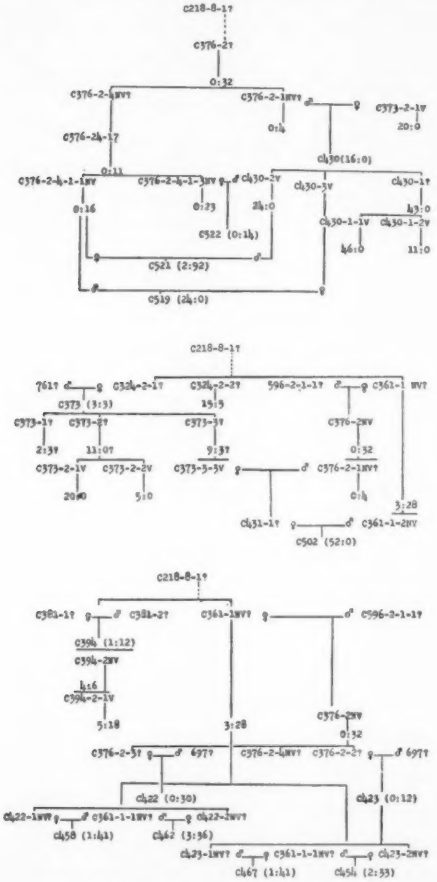
Inheritance of variegation in tomatoes. V = variegated; NV = nonvariegated; ? = doubt as to the phenotype.

PARENT		PROGENY			
PEDIGREE	PHENOTYPE	YEAR RECORDED	GENERATION	V	NV
C373-1	?	1939	F_2	2	3?
C373-2	?	1939	F_2	11	0?
C373-3	?	1939	F_2	9	3?
C373-2-1	V	1940	F_3	20	0
C373-2-2	V	1940	F_3	5	0
C324-2-1	?	1939	F_3	25	10
C324-2-2	?	1940	F_3	15	10
C430	V×NV?	1940	F_1	16	0
C519	V×NV	1941	F_1	24	0
C536	V×NV	1942	F_1	32	0
C430-1	?	1940	F_2	43	0
C430-2	V	1941	F_2	24	0
C430-1-1	V	1941	F_3	46	0
C430-1-2	V	1941	F_3	11	0
C502	?×NV	1941	Backcross	52	0
C521	NV×V	1941	F_1	2	92
C522	NV×V	1941	F_1	0	14
C394	?×?	1939	F_1	1	12
C394-2	NV	1940	F_2	4	6
C394-2-1	V	1941	F_3	5	18
C361-1	NV?	1940	F_2	3	28
C454	NV?×NV?	1940	F_1	2	33
C458	NV?×NV?	1940	F_1	1?	41
C462	NV?×NV?	1940	F_1	3	36
C467	NV?×NV?	1940	F_1	1	41
C376-2	NV	1939	F_2	0	32
C376-2-1	NV?	1940	F_3	0	4
C376-2-4-1	NV?	1940	F_4	0	11
C376-2-4-1-1	NV	1941	F_5	0	16
C376-2-4-1-3	NV	1941	F_5	0	23

One of the F_1 parents, C430-2, and all its F_2 progeny were extremely variegated and highly unfruitful. The phenotype of C430-1 is uncertain, as this plant was grown under glass and drastically pruned. Two V F_2 plants, C430-1-1 and C430-1-2, gave, in F_3, only V progeny. A V×NV F_1 backcrossed with NV gave 52 V plants (C502, fig. 4). No NV plants occurred in any of these F_2, F_3, or backcross families.

In 1941, two F_1 families, C521 and C522 (fig. 3), which were from NV ♀ parents of the same family, crossed with the same very much variegated plant, C430-2, contained 106 NV and only two slightly V plants. The two NV ♀ parents, C376-2-4-1-1 and C376-2-4-1-3, belonged to a family of

11 NV plants; and C376-2-4, the grandparent of this family, grown in 1939, was probably nonvariegated (fig. 3). V plants did occur, however, in the progeny from selfing a P_1 grandparent, C361-1, of this F_2 (fig. 5), and



FIGURES 3–5. (Figure 3 top, figure 5 bottom).—Pedigrees of tomato plants. The ratio of variegated to nonvariegated plants is shown under the series number of the parent plant or in parentheses after the series number of an F_1 family. "V" indicates a variegated parent; "NV" indicates a nonvariegated parent; "?" indicates some doubt as to parent phenotype.

it is possible that variegation was introduced by the ♀ parents of crosses C521 and C522.

These later, more complete data do not indicate that variegation depends on a dominant gene or genes, since there is no evidence of segregation

of genes for variegation in the F_2, F_3, and backcross families. Apparently, all or nearly all the megaspores of a variegated plant, but very few of the pollen grains, transmitted variegation. The most probable hypothesis seems to be that variegation is inherited through the cytoplasm and is, as a rule, maternally inherited.

Several families other than C521, which has already been mentioned, contained a minority of V plants. The phenotypes of most of the parents are not known. Many were grown in 1939, when slight variegation is likely to have been overlooked. In 1939, an F_1 family, C394, from unknown parent phenotypes, contained one V and 12 NV plants (fig. 5); and an F_2 from an NV F_1 parent, C394-2, contained a slight excess of NV plants. An F_3 from a V F_2 plant, C394-2-1, contained five V and 18 NV plants. Plant C361-1 (fig. 5), which was heterozygous for a deficiency and very slow-growing (Lesley and Lesley 1941), was probably nonvariegated. When this plant was selfed, only three of its 31 progeny were variegated. Four F_1 families from crosses C454, C458, C462, and C467 (fig. 5), whose parents are supposed to have been nonvariegated, also contained a minority of V plants. They had one parent in common, C361-1-1, which had V sibs; the other parents were also related to the deficient plant, which, as noted above, had a few V progeny. All the families containing a minority of variegated plants therefore originated from parents which were either variegated or related to variegated plants. Very slight variegation affecting only a few cells may easily have been overlooked in the supposed NV plants. On the whole, the evidence suggests that slightly V or even NV parents, when selfed or crossed with other NV plants, may give both NV and V progeny.

DISCUSSION

The palisade layer, which lies just beneath the upper epidermis of a normal leaf, is largely replaced in the abnormal portions of variegated leaves by irregular cells which resemble the spongy parenchyma.

A similar lack of palisade layer was observed by FUNAOKA (1924) in the pale portions of variegated *Acer Negundo* and *Glechoma hederaceum*. CONTI (1934), in a study of 37 plant species having typical hereditary white mottling, found that where chloroplasts were absent, there was a marked modification of the palisade layer in 18 species and a classical palisade structure in the other 19.

In the variegated tomato, the presence of small islands or isolated strips of normal cells in otherwise abnormal tissue, or *vice versa*, suggests that the change may proceed in either direction.

According to KÜSTER (1903, 1927), palisade tissue may be lacking as a result of shading, growth in artificial illumination, growth in extreme humidity or with leaves submerged, or as a result of fungal infection.

EAMES and MACDANIELS (1925) state that in normal plants the number of palisade layers and the density of the cell structure depend largely, either directly or indirectly, on light intensity. There may thus be a great variation in the proportion and arrangement of the palisade parenchyma in the same species growing under different conditions. There may also be considerable variation in mesophyll structure of leaves from different parts of the same plant. Since one part of a variegated tomato leaf may lack palisade cells while an adjacent region is normal, and since the abnormal condition is multiplied and transmitted somatically and is inherited, the condition which produces it must be within the cell and cannot be attributed to these external factors. The abnormal cytokinesis of the pollen mother cells on variegated plants seems to indicate that the cytoplasm is in an abnormal condition or that normal control over the cytoplasm is lacking.

According to LANGE (1927), in Solanum the germ cells originate from the subepidermal layer. The abnormality of this cell layer in the variegated tomato is certainly hereditary. That inheritance is predominantly maternal is indicated by the fact that no normal plants occurred in several carefully examined F_2 and backcross families from variegated ♀ parents. The variable proportions of variegated and nonvariegated plants found in some families and the instability of the variegation itself as it occurs on the plant also favor this hypothesis. The fact that variegation occurs earlier and develops more extensively in the progeny of a much-variegated seed parent than in that of a slightly variegated parent, is suggestive of a variegation inherent in the cytoplasm. Nevertheless, a gene interpretation is not excluded. Nontransmission by the pollen might be due to a pollen-lethal gene, and the scarcity of nonvariegated plants in the F_2 and backcross families might be due to the existence of several dominant genes for variegation. It is possible, also, that the normal F_1 plants from crosses C521 and C522 (fig. 3) would give variegated plants in F_2.

Since the flowers of predominantly pale shoots are sterile, variegated plants are usually reproduced by seeds from mixed or normal-looking shoots. Variegated plants apparently develop from all the egg cells, even of a ♀ parent of which some shoots appear to be nonvariegated. Because of somatic segregation, it may be possible to increase or decrease the amount of variegation by bud selection. At present the most promising method of eliminating variegation from a variety is by crossing it with a nonvariegated race and by repeated backcrossing, the variegated variety being used as pollen parent.

The plastids are the only known agents in cytoplasmic inheritance. Some relation between variegation and plastid distribution was accordingly suspected. In the subepidermal cells of the pale areas of the leaf, there are probably fewer chloroplasts than in the palisade cells of a normal area. In

the pale portions of the stem, the cells of the subepidermal layer are modified in shape and lack both chloroplasts and anthocyanin.

CHITTENDEN (1927) adduces evidence that, in some cases of mosaic anthocyanin distribution, the presence of anthocyanin depends on the activity of the plastids, and that in such plants maternal inheritance through plastids is more probable than inheritance by genes. In the variegated tomato inheritance of mosaic anthocyanin distribution appears to be cytoplasmic, and the pale-green color and lack of purple anthocyanin seem to depend on a deficiency of chloroplasts. Possibly the lack of anthocyanin is caused by insufficient glucose, two molecules of which enter into the formation of anthocyanin.

It appears that the presence of purple anthocyanin is determined by the independent genes *A*1 and *a*2 (MACARTHUR 1934) and by the chloroplasts. In the tomato chimera with variegated fruit, described by MACARTHUR (1928), which seems to be a typical case of plastid inheritance, purple anthocyanin color is not affected. However, the modified cell form of the subepidermal layer in abnormal regions of both stem and leaf, the abnormal cytokinesis in the pollen mother cells, and the fact that green plastids are present in the subepidermal cells of the leaf, suggest that in the present instance, variegation is due to an unstable or a "diseased" condition of the cytoplasm, as postulated by CORRENS (1937), which affects the formation of chlorophyll and anthocyanin. It seems probable that a certain specific portion of the egg cytoplasm may form the subepidermal layer and is abnormal in contrast to the rest of the cytoplasm.

SUMMARY

A variegation of tomatoes is described, in which abnormal pale-green areas occur on normal green stems and leaves. The pattern of pale-green and normal tissues on a variegated plant is very irregular, and the pale-green area is extremely variable in extent. On a predominantly pale shoot, flower development is arrested, cytokinesis is irregular, and the pollen is very scanty. In extreme cases the entire plant is unfruitful. Variegated plants which have the purple anthocyanin gene *A*1 have no anthocyanin in the pale portions of the stem and very little in the petioles of pale leaves.

In the pale portion of the leaf, palisade cells are absent, so that the upper surface is similar in color to the lower leaf surface. In the stem, the cells of the subepidermal layer of the chlorenchyma are abnormally flattened and contain no chloroplasts.

The variegation was not transmissible by the methods ordinarily effective with a virus and did not spread to adjacent unrelated families.

Variegation behaves as a dominant character. It is, as a rule, inherited maternally and appears to have its basis in the cytoplasm. Some parents

which were not known to be variegated gave a small proportion of variegated progeny. The occurrence of about two percent of variegated plants from nonvariegated ♀ × variegated ♂ may therefore be due to variegation introduced by female parents in which variegation was overlooked.

The absence of chloroplasts in the subepidermal layer of the stems of variegated plants may account for the lack of anthocyanin in the abnormal areas. The histological and cytological changes found in the pale portions of variegated plants, and the mode of inheritance of variegation, indicate that variegation is due to an abnormality of the cytoplasm.

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